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Water Quality in Open Finished Water Reservoirs Allegheny County, Pennsylvania

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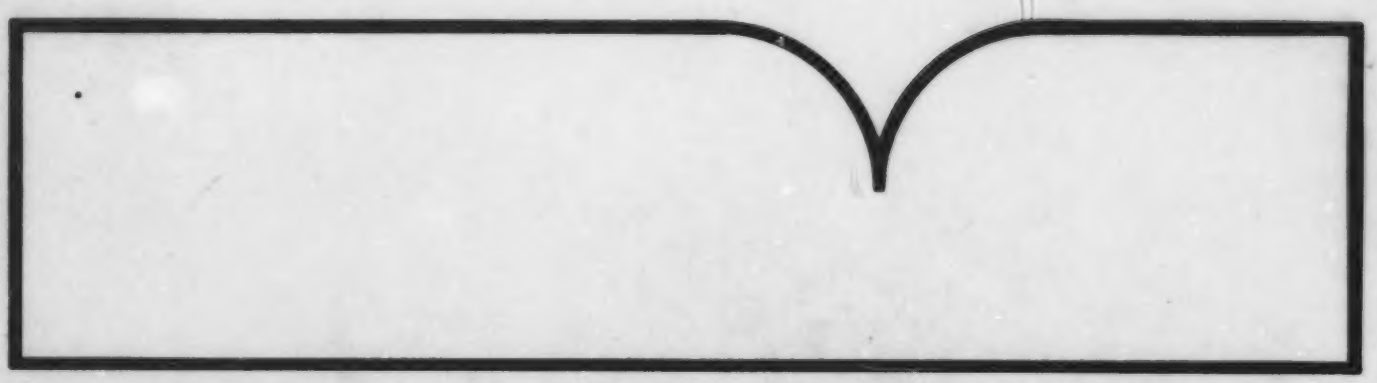

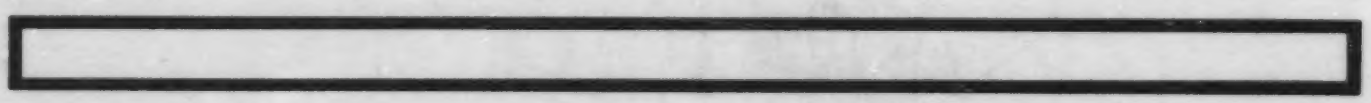

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WATER QUALITY IN OPEN FINISHED WATER RESERVOIRS - ALLEGHENY COUNTY, PENNSYLVANIA

by

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16. ABSTRACT

The purpose of this investigation was to study water quality changes occurring in open reservoirs in the distribution systems of five water supplies located in Allegheny County, Pennsylvania. Results of chemical, bacteriological, and biological analyses showed deterioration of water quality in the reservoirs, and a significant improvement in water quality occurred after two of the reservoirs were covered. A highly significant relationship was observed between density of autotrophic organisms and endotoxin concentration in the open reservoirs, and a similar relationship between total bacterial population (as measured by the standard plate count with an extended incubation period of 8 days) and endotoxin concentration.

Chlorella vulgaris was the most common autotrophic organism identified and it was found to produce a nonspecific reaction in the Limulus amoebocyte lysate (LAL) assay. Schizothrix calcicola was the most common Cyanobacterium observed and this species produced true LAL gelation indicating the presence of endotoxin. Bioassays have shown S. calcicola to be toxic to mice when injected intraperitoneally and the toxin was characterized as Slow Death Factor (SDF) producing mortality within 24 hours.

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FOREWORD

The primary mission of the Health Effects Research Laboratory is to provide data which is based on health-related research to support the regulatory activities of the Environmental Protection Agency. Research is conducted to identify, characterize, and quantitate the harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents in the environment. Research data are used in the Agency's standards setting procedure to insure that man and his environment are protected.

The objectives of the investigation reported herein were to identify water quality changes in six open reservoirs in the distribution systems of five water supplies in Allegheny County, Pennsylvania. There are over 700 locations in this country where water is treated to assure that it is safe for human consumption and then piped into the distribution system where storage is provided in an open reservoir. Water stored in this manner is subject to contamination from a number of sources and E.P.A. has recommended that open reservoirs should be protected by covering.

Results of the investigation showed chemical, bacteriological, and biological degradation of water quality in the reservoirs and a highly significant correlation between algal concentrations and a positive response in the Limulus amoebocyte lysate assay. Further laboratory study of the positive response indicated that green algae, which were dominated by Chlorella vulgaris, produced a false positive reaction while the blue-green algae, dominated by Schizothrix calcicola, produced a true reaction indicating the presence of endotoxins. The human health effects of the concentrations of endotoxins encountered in the investigation are unknown at this time.

Two of the reservoirs were covered while this study was in progress and it provided investigators with the opportunity to compare water quality data pre- and post-covering. Installation of the covers resulted in substantial water quality improvement. These results reinforce those from a previous study (An Investigation of the Effect of Open Storage of Treated Drinking Water on Quality Parameters, EPA-600/1-77-027, May 1977) and provide additional justification for the recommendation that open reservoirs in water distribution systems be covered.

R. J. Garner
Director
Health Effects Research Laboratory

ABSTRACT

The purpose of this study was to evaluate the distribution of algae, investigate sources and occurrence of endotoxins (lipopolysaccharides, LPS), and provide data on toxicity of Schizothrix calcicola using five drinking water systems located in Allegheny County, Pennsylvania, examined during the spring and summer of 1977-78. Results of chemical, bacteriological, and biological analyses have shown that highest concentrations of phytoplankton and bacteria were in open finished water reservoirs. The Limulus amoebocyte lysate (LAL) assay revealed a highly significant correlation between density of autotrophic organisms and "endotoxin" concentrations in open, finished water reservoirs which resulted from a nonspecific reaction between algae and LAL. A similar, though slightly less significant, relationship was observed between Standard Plate Count (SPC) bacteria and LAL gelation. Chlorella vulgaris, the most common autotrophic organism occurring in the five water systems, was found to be the major cause of nonspecific LAL reaction. S. calcicola was the most common Cyanobacterium in the examined drinking water supplies. This species produced "true" LAL gelation. Extraction of S. calcicola with hot phenol water indicated that this organism contained LPS. Bioassays have shown this species to be toxic to mice when injected intraperitoneally, and the toxin was characterized as a Slow Death Factor (SDF) usually resulting in mortality within 24 hours.

Results obtained in this study permit a conclusion that there is a deterioration in water quality in open reservoirs and distribution systems. This deterioration of water quality could be eliminated by covering of the reservoirs. Evidence supporting this conclusion was obtained by comparison of water quality at the treatment plant and at the reservoirs.

This report was submitted in fulfillment of Grant No. R805368 under the partial sponsorship of the U.S. Environmental Protection Agency. This report covers a period from March 1, 1977 to March 31, 1979, and work was completed as of June 30, 1979.

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SECTION I

INTRODUCTION

A waterborne outbreak of gastroenteritis affected approximately 5,000 persons in Sewickley, Pennsylvania, during August 1975. Extensive analysis of medical and environmental samples failed to identify a causative agent, however, investigation of the Sewickley water system revealed an accumulation of Schizothrix calcicola (Cyanobacteria) in open, finished water reservoirs (Lippy and Erb, 1974). One month after the outbreak, the water in one reservoir was still contaminated by this species (400,000 cells ml^{-1}) and by high endotoxin concentration (2,500 ng ml^{-1}).

The occurrence of large concentrations of Cyanobacteria and endotoxins in drinking water coinciding with the outbreak of waterborne gastroenteritis were the major factors leading to this investigation. On this basis the following objectives of the study were developed: (1) to evaluate the occurrence and distribution of algae in water systems with open reservoirs, (2) investigate sources and occurrence of endotoxins in drinking water, and (3) provide data on toxicity of S. calcicola.

Toxic algae and Cyanobacteria are found in diverse aquatic environments and have been implicated in the death of fish, wildlife, domestic animals, and humans (Moore, 1977). Many incidents of animals and even human poisoning caused by blue-green algae have been reported by Schwimmer and Schwimmer (1964, 1968) in their exhaustive review. The majority of the observed toxicities were caused by ingestion of Cyanobacteria from reservoirs, lakes, and ponds. Reports of blue-green bacteria toxicity have come from many different parts of the world and different aquatic environments. They emphasize the cosmopolitan distribution and great adaptability of toxic strains of Cyanobacteria.

Tisdale (1931) and Veldee (1931) mentioned that unusually heavy blooms of Cyanobacteria in water supplies might have been responsible for an epidemic of waterborne gastroenteritis in the drainage basin of the Ohio and Potomac Rivers during the drought year of 1930-31. Dean and Jones (1972) suggested that algal toxins could have been involved as a causative agent in a repeated and prolonged gastroenteritis episode in the Philippines. The occurrence and reoccurrence of diarrhea in Udaipur, India, has been attributed to the presence of Microcystic aeruginosa in drinking water (Gupta and Dashora, 1977). This study was preceded by the work of Aziz (1974) who isolated a nondialyzable toxin from M. aeruginosa which proved to be diarrheagenic to guinea pigs.

Seven of some 50 genera of freshwater blue-green bacteria, namely, Anabaena, Aphanizomenon, Coelosphaerium, Gloeotrichia, Microcystis,

Nodularia, and Nostoc include toxic species and strains. The toxins produced by the freshwater genera of blue-green bacteria were biochemically characterized as Anatoxins (Anabaena), Saxitoxins (Aphanizomenon), and toxic polypeptides (Microcystis) (Moore, 1977).

S. calcicola is not listed among the toxic species of Cyanobacteria. However, there is one publication which indicates that S. calcicola may form toxic strains. A chemical analysis of this species performed by Banner (1966) revealed one or more toxins in both benzene and water layers used in the extraction procedure, but he was unable to chemically characterize the Schizothrix toxins.

The toxicity of the planktonic strains of S. calcicola have not been investigated, and no attempts were made to isolate toxins from this form. S. calcicola has been found in a wide variety of environmental conditions and described as "perhaps the most widely distributed and common blue-green alga on earth" (Drouet, 1963). It has been discovered in hot springs, in melt-waters of Antarctica, in the Dead Sea at an elevation of 1,286 ft., as well as in the Himalayas at an altitude of over 17,000 ft. It is not surprising that S. calcicola can withstand adverse conditions in open, finished water reservoirs treated with chlorine and algicides. It is the most commonly found Cyanobacterium in drinking waters.

It has been shown by several authors that blue-green bacteria may contain macromolecular substances similar to lipopolysaccharides (LPS or endotoxins) of gram-negative bacteria. Endotoxin has been isolated from the following Cyanobacteria: Synechococcus (Anacystis) nidulans, Anabaena variabilis, Agmenellum quadruplicatum, Phormidium africanum, P. laminosum, P. uncinatum, and Schizothrix calcicola (Buttke and Ingram, 1975; Keleti et al. 1979; Mikheyskaya et al. 1977; Weckesser et al. 1974; Weise et al. 1970).

Generally, LPS consists of three distinct structural regions of differing properties; an O-specific polysaccharide (Region I) which is linked to the R-core polysaccharide (Region II) which is adjacent and linked to Lipid A (Region III). Lipid A, a long chain fatty acid derivative of a polyphospho-D-glucosamine, is an important part of the LPS molecule because it is responsible for most of the biological effects. The entire LPS structure affects biological systems and lipopolysaccharides are responsible for a number of physiopathological effects. The most widely studied response is pyrogenicity and endotoxemia in man characterized by a typical biphasic fever response. In addition, the endotoxins elicit biological and physiological responses such as leucopenia, leucocytosis, shock, Schwartzman phenomenon, depression of blood pressure, induction of interferon production, dissolution of cancerous tumors, and Limulus amoebocyte lysate gelation (LAL).

Until recently, the gram-negative bacteria were considered to be the major source of endotoxin. Jorgensen et al. (1976) speculated that a correlation may exist between bacteria and endotoxin in water. He also categorized the endotoxin occurring in water into three groups - total, bound, and free. Free endotoxin is present either in the Millipore filtrate

or supernatant of the centrifuged sample. Total endotoxin is determined directly in the analyzed sample, and bound (in cells) endotoxin constitutes the difference between the total and free endotoxins. Evans et al. (1978) has shown that the firm clot Limulus lysate procedure (LAL) produced significant correlation between total endotoxin concentrations and heterotrophic bacteria in samples collected from a small stream. An improved correlation with bacteria has been obtained by the use of bound endotoxin measured with the spectrophotometric technique. Similar results were obtained by Watson et al. (1977) from the marine environment.

It is interesting to note that Evans et al. (1978) found chlorination to cause a decrease in the bound endotoxin due to cell destruction which may affect the correlation with bacteria. Jorgensen (1979) supported this conclusion and suggested that the poor correlation he found between endotoxin content and densities of viable microorganisms in chlorinated renovated wastewater resulted from partial destruction of cells by end process treatments and varying degrees of solubilization of cell associated endotoxin prior to or during the treatments.

The effect of ingested LPS and its occurrence in drinking water has not been intensively studied and remains a subject of controversy. However, numerous unexplained cases of endotoxic reaction that have occurred in hospitals affecting mostly debilitated, immuno-suppressed patients, are suspected to have originated from the presence of endotoxins in the milk or water that was consumed by the victims shortly before the affliction developed (DiLuzio and Friedman, 1973).

More recently, it has been suggested that a possible relationship may exist between endotoxin levels in milk and the incidence of infant Sudden Death Syndrome (Crib Death) (DiLuzio and Friedman, 1973). This study indicated that a substantial increase of endotoxin levels occurred after prolonged exposure of the milk to room temperature. It was further noted that Crib Death is rare in breast-fed infants. Enhanced gastrointestinal permeability in infants suggests that absorption of exogenously derived endotoxin could initiate illness under certain conditions. Interestingly enough, the highest value of 800 ng ml^{-1} of LPS was detected by DiLuzio and Friedman (1973) in the tap water of Mexico City. This high endotoxin level does not seem to affect the indigenous population but may cause "traveler's diarrhea" of newcomers (tourists) to the area. Similarly, endotoxins may be responsible for diarrhea in weaned breast-fed infants who can be characterized as "newcomers" to a new environment.

The presence of endotoxins in drinking water and the effect of injected LPS have been observed by Hindman, et al (1975) while studying an epidemic of pyrogenic reactions among kidney dialysis patients. This epidemic occurred simultaneously with the maximum concentration of algae in the raw water source. Recently, three cases of endotoxin reaction (fever, chills, and hypotension) were observed in patients undergoing cardiac catheterization (MMWR, 1979). The investigation suggested that the endotoxins were induced by contaminated distilled water used for cleaning the catheters. The

difficulty with LPS is that most of the standard sterilizing procedures used in hospitals effectively destroy organisms but leave endotoxin residuals, because they are relatively heat stable.

The studies conducted by DiLuzio and Friedman (1973); and Jorgensen, et al. (1976) have demonstrated the feasibility of using the LAL procedure for the quantification of endotoxin levels in drinking water. The investigators have discovered elevated LPS levels in drinking water originating from surface sources while lower concentrations were recorded from systems treating groundwater. In spite of this effort, no attempt was made to test reliability of LAL for drinking water examination nor were the sources of LPS discussed and explored.

The oral ingestion of endotoxins produced by algal and/or cyanobacterial blooms may be responsible for some of the waterborne disease outbreaks presently classified as "of unknown etiology." The presence of high concentrations of endotoxins and S. calcicola in the Sewickley, Pennsylvania, water system shortly after a massive outbreak of waterborne gastroenteritis of unknown etiology indicated a possible relationship between the consumption of endotoxins and illness (Sykora, et al., 1977; Lippy and Erb, 1976).

This report examines distribution of algae in drinking water, explores the sources of LAL gelation in conjunction with toxicity, and investigates the possible endotoxin isolation from S. calcicola.

SECTION II

CONCLUSIONS

1. Results of the field study of five water systems showed that microbiological populations increased and water quality deteriorated when water was stored in open reservoirs. These results were less notable where chlorine residuals were maintained or partially maintained through reservoir storage.

2. Chlorella vulgaris, a green alga, generally dominated phytoplankton populations during the two-year study of open reservoirs. Cyanobacteria, of which Schizothrix calcicola was the most common species, dominated in only one reservoir and were apparently controlled by chlorination since S. calcicola was reduced in the distribution system and C. vulgaris again became the dominant phytoplankton organism. This occurrence was observed during 1978 in Highland Park Reservoir No. 1 in the Pittsburgh water system. The greatest cell count of S. calcicola during this period was 5,000 cells ml^{-1}.

Study of the five water systems indicates that the water quality conditions recorded during the Sewickley Outbreak in 1975 were unusual. The occurrence of S. calcicola as the predominant Cyanobacterium and its concentration (400,000 cells ml^{-1}) was markedly different when compared with results of this study.

3. Coliform bacteria absent in samples of water from the treatment plants, were occasionally found in samples collected from the open reservoirs and rarely detected in the distribution systems, following reservoir storage. Standard Plate Counts and Gram-Negative Bacterial Counts followed a general pattern of low densities at the treatment plants with substantial increases after open reservoir storage and a subsequent decrease in population density in samples obtained from the distribution systems.

4. Current microbiological standards enforceable under the Safe Drinking Water Act are limited to coliform bacteria and require a representative number of samples from the distribution system to be collected on a monthly basis. Water systems with open reservoirs should not rely on this standard alone to assure acceptable microbiological water quality.

5. Subsequent installation of floating covers on the Sewickley Reservoirs has resulted in an improvement in water quality. There was a substantial reduction in SPC bacteria, endotoxins and phytoplankton, and chlorine residuals increased. A problem was detected with the type of access hatch that was used. The hatch design permitted pooling of rainwater and subsequent contamination by coliform organisms. This can be corrected by an improved hatch design.

6. Endotoxin concentrations determined by LAL assay were substantially higher in open reservoirs than in water produced at the respective treatment plants. There was a significant correlation in LAL titer with algae and SPC bacteria incubated at 35°C for eight days. Statistical and graphical analyses of the data indicate that LAL gelation in open finished water reservoirs is predominantly determined by the concentration of algae and Cyanobacteria.

7. Experiments performed with C. vulgaris cultures showed that green eukaryotic organisms are responsible for nonspecific LAL gelation which cannot be characterized as a "true" endotoxin reaction. Treatment of C. vulgaris cultures with lysozyme, which destroys peptidoglycans and possibly other similar compounds reported to react with LAL, removed the gelling capability showing that this species does not produce "true" endotoxin.

Experiments performed with S. calcicola indicated the presence of "true" lipopolysaccharides in this blue-green alga. Samples from cultures of this species subjected to lysozyme treatment continued to produce LAL gelation. When the cultures were subjected to alkaline application, which destroys LPS, the LAL reaction was negative. The LAL response continued to be positive for C. vulgaris after alkaline exposure indicating nonspecific LAL reaction with this species of alga.

8. S. calcicola fresh culture was toxic to mice when injected intraperitoneally with LD_{50} levels ranging from 90-385 mg kg^{-1}. Toxicity experiments with Anabaena flos-aquae using 8-day old cultures as positive controls indicated almost identical LD_{50} levels as that of S. calcicola. Fresh, concentrated cultures of S. calcicola intubated into mice resulted in insignificant mortality. Concentrations higher than 9 g kg^{-1} were so dense that they could not be used for intubation. Therefore, the LD_{50} for intubated S. calcicola was estimated as > 9 g kg^{-1}. The autopsy results support the fact that S. calcicola is somewhat toxic even when intubated into mice. The LD_{50} of intubated 8-day old cultures of A. flos-aquae was approximately 10 times lower than that obtained from IP injections.

Death caused by A. flos-aquae cultures was always observed within several minutes whereas mortality due to S. calcicola occurred within 24 hours. The heat labile toxin of S. calcicola can be characterized as a SDF which is probably produced by bacteria closely associated with S. calcicola. The other factor which may contribute to the toxicity of S. calcicola is the endotoxin which was detected in this species by LAL gelation and confirmed by alkali and lysozyme treatment and by a preliminary phenol extraction.

SECTION III

RECOMMENDATIONS

1. A study of open reservoirs in five distribution systems in Allegheny County produced results which showed a deterioration in chemical and microbiological water quality. When two of the reservoirs under study were protected with a floating cover, an improvement in water quality occurred with a reduction in microbial populations and the maintenance of a chlorine residual. Open finished water reservoirs should be covered to maintain water quality and prevent contamination from exterior sources.

2. There are no monitoring requirements or biologic standards for algae in water systems. Current microbiological standards enforceable under the Safe Drinking Water Act are limited to coliform bacteria and require a representative number of samples from the distribution system on a monthly basis. Results of this study showed the absence of coliform bacteria, which would indicate safe and acceptable water quality, while other biological indicators showed degraded and, in some cases, unacceptable water quality.

Additional monitoring requirements and biological indicators are required for water systems, especially those with open finished water reservoirs.

3. Laboratory studies demonstrated toxicity of S. calcicola and A. flos-aquae in mouse assay. Additional study is necessary to determine the implications of blue-green bacteria in drinking water.

4. LAL technique is a nonspecific assay which indicates true endotoxins, but may also be affected by the presence of green algae and gram-positive bacteria. In spite of its deficiencies, LAL gelation is a good indicator of biological contamination of water and can provide results in a short period of time (within hours after collection of the sample). Thus, LAL assay can be a useful technique in tracing the source of contamination when time is limited and could be utilized during waterborne disease investigation. A special study design to determine the accuracy and predictive power of the LAL technique with respect to total, bound, and free endotoxins for bacteria and algae quantification in drinking water is recommended. This study would utilize the alkali and lysozyme treatment to determine the origin of LAL gelation. A comparison of the firm clot method with spectrophotometric measurement technique should be included.

SECTION IV

MATERIALS AND METHODS

The study was conducted in two phases. In the first phase, a field study was undertaken to determine the concentrations of endotoxins and selected bacteriological and chemical characteristics of five water systems, with emphasis on water quality in open finished water reservoirs. In the second phase, laboratory experiments were conducted on three phytoplankton species to determine their toxicity to mice and reaction with LAL. Two of these species were determined by field survey to be the major contaminants of the reservoirs that were studied.

FIELD STUDY

Water Supply Systems

Five water systems in Allegheny County, Pennsylvania, area were selected for study in this phase. Each of the systems uses open distribution reservoirs for storage of finished water.

The water systems studied were:

a. Dixmont State Hospital
b. City of McKeesport
c. City of Pittsburgh
d. Borough of Sewickley
e. Borough of Springdale

The systems were chosen on the basis of their source of raw water, the presence of open reservoirs within their distribution system, and whether water quality problems occurred in the past. Basic technical data are summarized on Table 1 and additional information is provided below.

Springdale

Four wells that penetrate an unconfined aquifer adjacent to the Allegheny River are the source of supply. The wells are drilled to a depth of 18.3 meters (60 feet). Raw water is pumped from the wells to a treatment plant where simple ion-exchange softening reduces the hardness from 190 to 10-25 mg l^{-1} as $CaCO_3$. The softened water is blended or mixed with raw water that is allowed to bypass the process in order to attain an optimum hardness of 20 mg l^{-1}. After the softening process, the PH is adjusted by addition of caustic soda, chlorine gas is injected to provide disinfection, and the treated water is delivered to a clearwell.

TABLE 1. BASIC TECHNICAL DATA OF FIVE ALLEGHENY COUNTY WATER SYSTEMS

System	Source	Treatment Train*	Output (MGD)	Population Served	Reservoirs Examined	Reservoir Vol. (MG)
Sewickley	Groundwater	$PreCL_2$→Soft→ RSF→$PoCl_2$	1.80	8,000	SW†	5.0 1.8
Dixmont	Ohio River	C→S→RSF→$PoCl_2$	0.24	950	DXS‡	1.0 0.7
McKeesport	Youghiogheny River	C→S→ACF→$PoCl_2$	10.00	59,000	MCK	7.5
Springdale	Groundwater	Soft→pH→$PoCl_2$	0.59	6,500	SPR	0.5
Pittsburgh	Allegheny River	$PreCl_2$→C→S→ RSF→$PoCl_2$	65.00	450,000	HP†††	177.5

* $PreCl_2$ = Prechlorination, Soft = Softening, pH = pH Adjustment, C = Coagulation, S = Sedimentation, RSF = Rapid Sand Filtration, ACF = Anthracite Coal Filter, $PoCl_2$ = Post Chlorination

† Sewickley Reservoirs Nos. 3 and 4

‡ Dixmont Reservoir South

††† Highland Park Reservoir No. 1

Water is pumped from the clearwell to the distribution system and an uncovered reservoir with a storage capacity of 1895.7 m^3 (0.5 MG) and a surface area of 566.7 m^2 (6100 square feet). The reservoir is located 1.9 km (1.2 miles) from the treatment plant on an isolated hilltop in Springdale Township. It is situated in a pine grove and surrounded by a 3 meter (10 feet) high fence topped with barbed wire. The reservoir is used to supply the distribution system by gravity between 4:00 p.m. and 7:30 a.m. when pumps at the treatment plant are not operated. While the water is in the reservoir, it does not undergo any chemical treatment. Occasionally, the reservoir will be purposely overflowed to clean out the floating debris which has accumulated.

McKeesport

Water is pumped from the Youghiogheny River to the treatment plant which has a capacity of 32,852 m^3/day (10 MGD). Raw water enters the plant as a large turbulent fountain to which alum, ferric sulfate, and lime are added. Periodically, powdered activated carbon in a slurry is added for taste and odor control. Following coagulation the water is chlorinated, settled, and filtered through granular anthracite coal. Filtered water flows to clearwells and is subsequently pumped to an open storage reservoir for distribution.

The 28,389.8 m^3 (7.5 MG) distribution reservoir is a concrete basin 7.6 meters (25 feet) deep located in the midst of the residential section of McKeesport. Water enters the reservoir at the surface and chlorine is added through a diffusion manifold on the bottom of the reservoir. The outlet to the distribution system is also at the bottom of the reservoir near the point of chlorination. In order to control algae occurring below the surface dry calcium hypochlorite is occasionally distributed along the edges of the reservoir but at no time is copper sulfate added to the water.

Pittsburgh

The Pittsburgh Water Treatment Plant receives raw water from the Allegheny River and treats an average of 246,044.5 m^3/day (65 MGD). Water is first delivered to clarifiers where it is pre-chlorinated. Lime and alum are subsequently added for coagulation and clarification. Permanganate is used for conversion of manganese and iron into an insoluble precipitate.

A coagulant aid is added to increase the efficiency of coagulation followed by the use of activated carbon to remove organic compounds that cause tastes and odors. Water then proceeds to sedimentation basins. Following sedimentation the water is processed through rapid sand filters and subsequently pumped to an underground storage reservoir. Here it is again chlorinated and the fluoride concentration is adjusted. Chlorine dioxide is added for disinfection and oxidation of phenolic compounds. Soda ash is provided for pH adjustment.

Finished water is then pumped to seven open reservoirs. This study concentrated on Highland Park Reservoir No. 1 which is the largest in the Pittsburgh system. This reservoir has a storage capacity of 444,772.8 m^3

(117.5 MG) and covers an area of approximately 0.24 km^3 (60 acres). The flow through this reservoir is 113,559 m^3/day (30 MGD) which yields a theoretical detention time of 4 days. The water leaving the reservoir is chlorinated.

During the months of May through October copper sulfate and calcium hypochlorite are added to the reservoir two or three times a week to control biological growths. The copper sulfate, in crystalline form, is poured into the water from a motor boat which circles the reservoir. As a result, the edges of the reservoir receive a heavy dose of copper sulfate (up to 10 mg l^{-1}). This results in the control of benthic algae which grow on the sloped edges and prevents the release of tychoplanktonic forms, which may contaminate the main body of water. The calcium hypochlorite (used for autotrophic organisms and bacteria control) is dispersed throughout the reservoir in pellets which sink to the bottom and slowly dissolve over a four-to-eight-hour period.

A tube aeration system installed in the reservoir is occasionally utilized for mixing and aids in dispersing chlorine released by the pellets to control biological growths in the reservoir.

Dixmont

Dixmont State Hospital operates its own water treatment plant which services a population of approximately 950. The treatment plant obtains raw water from the Ohio River. Chlorine, alum, soda ash, lime and activated carbon are added to the water during the course of treatment. Physical treatment includes flocculation, sedimentation and rapid sand filtration. Approximately 757 m^3 (0.2 MG) of finished water are produced every weekday. In 1977 some of the finished water was pumped to two uncovered storage reservoirs and the remainder to the distribution system. The water stored in these impoundments was utilized only during the night and on weekends during the time the plant was not operating. Since 1978 all the finished water is pumped to the reservoirs which feed the distribution system.

For this study, the samples were collected only from Dixmont No. 1 which is a 4163.8 m^3 (1.1 MG) uncovered reservoir. Immediately next to it is Dixmont No. 2, a 3785.3 m^3 (1 MG) uncovered reservoir. These reservoirs were typically storing about 2649.7 m^3 (0.7 MG) each during the study period.

Sewickley

Originally Sewickley obtained all raw water from an intake crib located beneath the Ohio River. Recently, a well was drilled and presently provides almost 60% of the raw water.

The capacity of the Sewickley treatment plant is 68,135 m^3/day (1.8 MGD) and treatment consists of prechlorination, softening-filtration, post-chlorination, fluoride adjustment, polyphosphate addition for manganese control, and the addition of caustic for pH adjustment and corrosion control.

Finished water is pumped from the plant directly to Reservoir No. 1. Reservoir No. 1 has a storage capacity of 18,926.5 m^3 (5 MG) and a theoretical residence time of approximately five days. From Reservoir No. 1 the effluent is pumped to Reservoir No. 3 which has a storage capacity of 6737.8 m^3 (1.78 MG) with a theoretical residence time of approximately 2.5 days. This reservoir supplies water by gravity to an area known as the low pressure distribution system. From Reservoir No. 3, the water is pumped to Reservoir No. 4 which has a storage capacity of 3785.3 m^3 (1 MG). It serves an area known as the high pressure distribution system. The residence time in Reservoir No. 4 is supposed to be brief because of the configuration and proximity of inlet to outlet (Lippy and Erb, 1976). The reservoirs are located in rugged and steeply sloping terrain and are surrounded by trees.

Copper sulfate was used regularly to control algal growth in all three reservoirs while they were still open. During the summer, all the reservoirs were usually treated once a week at a dosage to maintain 1.0 mg Cu l^{-1} in each reservoir. After the 1975 waterborne disease outbreak incident, additional chlorination was installed between Reservoir No. 1 and 3 which increased the residual chlorine in the distribution system and reservoirs. However, the chlorine levels in No. 4 remained very low.*

Sampling and Analytical Procedure

Collection of Samples

The systems were sampled from April 2 through July 12, 1977 and from June 12 through September 28, 1978. A total of 311 sets of samples was collected and analyzed on a weekly basis from each water system at the following locations:

1. Treatment plant effluent (TPE)
2. Open Reservoir
3. Distribution system

Water samples were collected from water treatment plants; open distribution reservoirs at the edge of the reservoir (farthest point from the inlet); and from the distribution system at permanently selected points. All the samples were placed on ice for preservation during transit.

Physical, Chemical, Bacteriological and Biological Measurements

Water samples were analyzed for chlorine concentration, pH, temperature, total hardness, SPC, concentration of algae and Cyanobacteria, and endotoxins. The analyses for nutrients (orthophosphates, ammonia, nitrites, and nitrites) were performed only during the 1977 sampling period. Analyses for fecal and

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*Originally, Reservoir Nos. 1, 3 and 4 were open but they were protected during the summer of 1978 with floating covers. Reservoir No. 4 was covered four weeks, and No. 3 six weeks after initiation of sampling.

total coliform bacteria were added in 1978.

Determinations of free and total chlorine were performed in the field immediately upon collecting the sample using the Taylor Chlorine Field Kit (DPD), while pH was measured in the laboratory using ORION ® pH meter equipped with a glass electrode. Temperature measurements were made in the field in conjunction with tests for chlorine residuals.

The measurement for orthophosphates, ammonia, nitrites, and nitrates were obtained using a Hach DR ® (Direct Reading) Colorimeter. The orthophosphate level was determined using the StannVer method. The ammonia level was determined using the Nessler method. The nitrite concentration was performed using the NitriVer III method while the nitrate level was obtained using the NitraVer V method. Conversion factors were applied when needed. In the case of orthophosphate, a calibration curve derived from laboratory standards was applied to the obtained readings.

The sampling procedure and determination of total hardness, Standard Plate Count bacteria, fecal and total coliform (membrane filter technique) bacteria were those described in Standard Methods for the Examination of Water and Wastewater, 14th Edition (1975). During 1978 sampling period the SPC bacteria, were incubated not only at 35°C but also at 20°C and colonies were counted after 48 hours and 8 days. The delayed SPC incubation was performed to detect slow growing bacteria. For the gram-negative bacteria identification, all the SPC colonies were characterized according to their apparent morphology, size, color and location (depth). All the colony types were tested using the standard gram staining. All bacteriological samples were collected and analyzed in duplicate. The two results were simply averaged in order to obtain a representative value.

Phytoplankton samples were collected in one liter bottles and preserved with Lugol's solution. After a settling period of several days (minimum of 72 hours), the samples were concentrated to 50 ml by siphoning off the supernatant. One to three aliquots of the concentrate were then quantitatively analyzed in a Palmer-Maloney nannoplankton cell. The resulting phytoplankton concentrations for the three aliquots were averaged and rounded to the nearest whole number. Individual cells were enumerated in those forms which are multicellular or colonial.

Limulus Amoebocyte Lysate Assay

The Limulus lysate (LAL) assay was used for endotoxin detection and quantitative evaluation. Samples for LAL analyses were collected in sterile pyrogen-free, test tube (Falcon) and were immediately placed in an ice chest for delivery to the laboratory where all samples were stored at 4°C until assayed. Analyses were conducted within one week of collection and freeze-dried Limulus lysate (Pyrotest ®, Difco Laboratories) theoretically capable of detecting as little as 2.5 ng ml^{-1} of endotoxin was utilized. Samples were adjusted to pH between 6-8 using pyrogen free 0.1 N hydrochloric acid and 0.1 N sodium hydroxide (Sigma Chemical Company, St. Louis, Missouri).

In all pH measurements, portions of the sample were decanted into 10 ml beakers for analysis. Following pH analysis this portion of the sample was discarded.

The LAL firm clot procedure for determination of total endotoxins was modified after that of Jorgensen et al. (1976). Water samples and the lysate were equilibrated at room temperature prior to actual assay. All tests were conducted using sterile, pyrogen-free, 10 x 75 mm test tubes. The assays were performed by simply adding 0.1 ml of the reconstituted lysate to the tube followed by the addition of 0.1 ml of the water sample. Contents of the tubes were thoroughly agitated using an automatic Vortex mixer. All assays were incubated for 60 minutes at 37°C in a water bath. The tubes were covered with aluminum foil and were not disturbed during the entire incubation period. In order to determine the activity of each batch of the lysate, serial dilutions of the Difco positive control containing 2.5 ng ml^{-1} of Escherichia coli endotoxin were used. In addition, pyrogen-free Difco Pyrotrol ® and Travenol ® were utilized as negative controls to provide information on possible pyrogen contamination of the dilution water. Sterile, pyrogen-free technique was practiced throughout the procedure.

Following the incubation period the tubes were carefully inverted in order to determine presence of gelation. Only responses classified as strong gelation (solid clot, +++) or evident gelation (clot breaks upon inversion, ++) were interpreted as positive reactions.

The Limulus lysate is an expensive reagent. For this reason during the 1977 period only tenfold differences in endotoxin concentrations were measured while in 1978 all effort was made to pinpoint the exact endotoxin values present in each sample by performing additional dilutions. Therefore, in 1977 concentrations of endotoxins in the water samples were determined by performing only serial, exploratory tenfold dilutions of the original sample. In order to increase the accuracy, additional twofold dilutions of the highest concentration displaying positive reaction were performed during 1978 sampling period. For example, if the undiluted sample indicated a positive response and the 1:10 dilution was negative, twofold dilutions were prepared between the undiluted sample and 1:10 dilution. In 1977 the sensitivity of LAL given by Difco was used as a standard. The LPS concentration of each water sample tested in 1978 was calculated using the established sensitivity of the lysate multiplied by the reciprocal of the greatest dilution factor which gelled the lysate.

LABORATORY EXPERIMENTS

Algal and Cyanobacterial Cultures

Chlorella vulgaris (Chlorophyta) was isolated from Pittsburgh drinking water and brown planktonic strain of Schizothrix calcicola (Cyanobacteria) was obtained from Mosquito Creek Reservoir, Ohio. The strain was identical to the dominant Cyanobacterium which was found during the Sewickley outbreak in 1975. Its identity was confirmed by Dr. F. Drouet of the Academy of Natural Sciences in Philadelphia. Attempts to isolate S. calcicola from drinking water supplies under study in Pennsylvania were unsuccessful due to

low densities occurring at the time of laboratory experiments (winter, 1977). Anabaena flos-aquae (toxic strain NRC-44) was obtained from Dr. Wayne W. Carmichael, Wright State University, Dayton, Ohio and green strain (UTEX 1937) of S. calcicola was received from Dr. R.C. Starr Collection, University of Texas, Austin, Texas.

S. calcicola and C. vulgaris were maintained using Allen's medium, while A. flos-aquae was grown in ASM-1-Tricine medium (Carmichael and Gorham, 1974; James, 1974). All of the algal and Cyanobacterial species were mass cultured in 9 l, clear pyrex glass bottles, stored at 24°C, aerated with compressed air at a ratio of 100 to 250 cc per minute, and illuminated by "white" fluorescent light adjusted to a 16-hour cycle at 345 foot candles. Each bottle was pre-aerated before inoculation with 200 ml of pure starter culture.

The number and biomass of bacteria occurring in phytoplankton cultures were determined by plating serial dilution of these cultures on Trypticase Soy Agar in duplicates. The plates were incubated for 72 hours at 25° and 35°C. The average number of bacteria per ml was established by counting the colonies growing on the plates. The biomass was calculated utilizing Salmonella typhimurium as a standard. A series of dilutions in physiological saline containing this organism was plated on Trypticase Soy Agar medium and the number of bacteria present in the culture was obtained. A known aliquot of the same culture was lyophilized and the dry weight of bacteria was established. Based on this standard, the average count of bacteria in S. calcicola and C. vulgaris cultures constituted only 0.04% of the total biomass.

Lysozyme and Alkali Treatment

Two samples of C. vulgaris and S. calcicola were treated with three times crystallized egg white lysozyme (100 µg/mg) at pH 6.3, in 0.15 N saline kept at 37°C for 18 hours (Wildfeuer, et al. 1975).

Alkali treatment was accomplished using the technique described by Suzuki, et al. (1977). The material was suspended in pyrogen free 0.1 N sodium hydroxide solution which was kept in a sealed tube in a water bath at 70°C for 4 hours. After neutralization with pyrogen free hydrochloric acid the samples were subjected to the LAL gelation test.

Toxicity Experiments and Toxin Isolation

For toxicity testing the cultures were harvested using a DeLaval ® gyro-tester flow-through centrifuge. The suspension was washed once with distilled water in an International Centrifuge Model V® at 2,000 rpm for five minutes. The concentrated cells were further processed to a damp pellet with Millipore filter apparatus equipped with an 0.8 mu pore size filter. The damp pellets were weighted on an analytical balance. The test doses were prepared using wet weight but the final, dry weight of each concentration tested was established after drying in preflamed, preweighted crucibles for 60 minutes at 105°C. Several tests with Anabaena cultures were performed using lyophilized cells.

The toxicity of S. calcicola (brown strain) and A. flos-aquae was established by intraperitoneal (IP) injection and by intubation into female, white Swiss-Webster mice of 19 to 25 g body weight.

For IP injections fresh, concentrated cells were diluted with 0.9% physiological saline solution. The volume injected was constant for any one experiment but varied from 0.2 to 0.3 ml according to the average weight of the mice. Sterilized syringes were used throughout; all glassware was sterilized and pyrogen-free. Adequate controls, injected with saline solution and pyrogen-free water in volumes equal to that used in tests were performed to check the technique. All mice were observed closely for symptoms and survival times during the first hour to five hours after injection, and thereafter at intervals of eight hours for three days.

For the direct intubation of Cyanobacteria into the stomach of mice a blunt syringe needle equipped with a ball was utilized. For each Cyanobacterial age group there were three control groups of mice-control 0 was not intubated, control 00 was intubated with 1.0 ml of 0.9% physiological saline solution and control 000 was intubated with 1.0 ml of the culture supernatant. The day before intubation the test animals were weighted, grouped, and numbered. Food and water were withheld for 12 hours to be provided ad libidum on the morning of intubation. For the next seven days after intubation, observations included daily weights, diarrhea, changes in activity, and autopsies on dead animals.

Toxin Isolation

Preliminary isolation of LPS was performed from green strain of S. calcicola (UTEX 1937) which was readily available at the time. S. calcicola was obtained from the existing cultures, grown and harvested with the same technique as previously described.

For endotoxin isolation the phenol water extraction technique, as outlined by Keleti and Lederer (1974), was utilized. The method, modified for Cyanobacteria is as follows:

a. Twenty grams of S. calcicola (wet weight) were suspended in 350 ml water and heated to 68°C in a water bath. (Note: all water used in this procedure was pyrogen-free.)

b. 350 ml of 90% phenol (preheated to 68°C) was added to the algae suspension and vigorously stirred for 30 minutes

c. The hot phenol-water-algae mixture was cooled to 10°C in an ice bath.

d. The mixture was then centrifuged at 7000 rpm for 45 minutes. (This was accomplished in two 350 ml sections due to the limited volume capacity of the laboratory's centrifuge.)

e. The clear upper water layer was aspirated off. This water layer contained the LPS and nucleic acids.

f. To the brown phenol layer and the insoluble white residue, an additional 350 ml of water was added, stirred, and then heated to 68°C in a water bath for 30 minutes.

g. Steps c, d, and f were repeated then, and all aqueous layers were combined.

h. These aqueous layers (900 ml) were dialyzed against distilled water for three days, with the water being changed every 6 hours.

i. Using a rotary evaporator (40°C under reduced pressure) the water volume was reduced to approximately 100 ml.

j. The remaining volume was centrifuged for 15 minutes at 5000 rpm and the pellet was discarded.

k. The supernatant was then lyophilized to yield the crude LPS. This crude LPS was further purified using the modified Westphal, Jann (1965) techniques as follows:

   1. The crude LPS was resuspended in water to give a 3% solution. The LPS solution was then centrifuged at 40,000 rpm (105,000 xg) for 4 hours.

   2. The supernatant was discarded and the pellet resuspended in a small amount of water.

   3. This was centrifuged at 3000 rpm for 10 minutes and the pellet was discarded.

   4. The supernatant was lyophilized to yield 97% LPS and approximately 3% nucleic acids.

ANALYSIS OF RESULTS

In order to gain a better understanding of the general conditions which exist at any particular sampling location, arithmetic means and standard deviations were computed for each location's chemical data and the geometric means and 95% confidence intervals were computed for those parameters which exhibited differences of several orders of magnitude, e.g., bacteria, algae, and endotoxins.

Simple and multiple linear regression analyses were also performed, correlating endotoxin concentrations with standard plate count bacteria, total and free chlorine, and total algae. The endotoxin concentrations and the other variables were transformed into logarithmic values in an attempt to find the best fitting linear relationships. Pearson's correlation coefficients and significance levels were computed and listed in Tables 16-19. The 0.05 levels of significance were designated as the criterion for rejection. The identical tests were used by Watson et al. (1977) and Evans et al. (1978) for statistical evaluation of the relationship between endotoxin and bacteria.

The LD_{50} per kg was calculated using standard procedures (Keleti and Lederer, 1974) or obtained from graphical interpolation. The weight change of intubated mice was statistically evaluated using hierachy analysis of variance which included Pearson's correlation, two way classification analysis of variance (ANOVA) and student t-test.

SECTION V

RESULTS AND DISCUSSION

FIELD STUDY

In this section, results of chemical, bacteriological, and biological monitoring are tabulated, analyzed and discussed. The emphasis is on the biological and bacteriological characteristics of water in open reservoirs and the production of endotoxins which may be introduced into the distribution systems and create a public health problem.

Temperature and pH

The temperature of Sewickley and Springdale finished water was relatively constant and low, typically characteristic of a groundwater source. Sewickley Reservoir No. 3 maintained a low temperature throughout both sampling periods averaging 15.7° and 19.7° in 1977 and 1978, respectively (Tables 2 and 3). This had a definite effect on temperatures within Sewickley low pressure system which averaged only 17°C in 1977 and 19.5°C in 1978. The temperatures recorded in Springdale reservoir were higher than those observed in Sewickley No. 3 but lower than at the remaining localities. They ranged from 14.5° to 21.5°C in 1977 and were slightly higher in 1978. The average water temperatures measured at the remaining localities were slightly above 20° but not reaching 26°C.

The data show that the pH at most of the sampling stations was relatively constant which could be attributed to the pH adjustment practiced by the treatment plants to optimize coagulation and control corrosion.

The data indicate that the reservoirs with high algae count usually have a higher pH than finished water (Springdale, Dixmont, Sewickley No. 4). Algae use carbon dioxide in their photosynthetic activity and its removal from water is responsible for increased pH conditions. It is interesting to note that the average pH of the surface water of McKeesport reservoir was lower (7.6) than the average pH of both the incoming finished water (8.45 in 1977, 7.9 in 1978) and the water in the distribution system (8.2 in 1977, 7.9 in 1978). This reservoir is equipped with additional chlorination and according to the treatment plant personnel, the residual chlorine content in the surface water is around 0.3 mg l^{-1} during the night while it drops to near zero during the day. Evidently, due to the action of sunlight, the hypochlorous ion is chemically transformed into hydrochloric acid responsible for the decrease in pH levels.

TABLE 2. ARITHMETIC MEANS ($\bar{x}$) AND STANDARD DEVIATIONS (SD) OF CHEMICAL AND PHYSICAL PARAMETERS -1977 DATA

Locality	Temperature °C $\bar{x}$	S.D.	pH $\bar{x}$	S.D.	Free Cl mg l^{-1} $\bar{x}$	S.D.	Total Cl mg l^{-1} $\bar{x}$	S.D.	Hardness $CaCO_3$ mg l^{-1} $\bar{x}$	S.D.	Phosphates PO_4 mg l^{-1} $\bar{x}$	S.D.	Nitrates NO_3 mg l^{-1} $\bar{x}$	S.D.	N
Springdale TPE	15.6	1.7	7.7	0.19	0.52	0.11	0.57	0.13	17	10.7	--	--	--	--	9
Reservoir	18.0	2.7	8.5	0.19	0.00	0.00	0.02	0.06	13.5	4.7	3.67	2.97	0.26	0.24	12
Distribution	22.1	2.0	7.7	0.15	0.28	0.12	0.34	0.14	16.6	12.0	--	--	--	--	9
McKeesport TPE	22.5	2.7	8.5	0.43	0.72	0.16	0.76	0.16	154.8	7.9	--	--	--	--	8
Reservoir	21.2	4.5	7.6	0.23	0.03	0.05	0.05	0.09	134.2	28.5	3.52	0.36	0.24	0.21	12
Distribution	21.6	1.9	8.2	0.33	0.60	0.13	0.67	0.11	159.3	13.9	--	--	--	--	9
Pittsburgh TPE	24.6	3.3	8.5	0.31	0.26	0.20	0.41	0.32	154.8	18.6	--	--	--	--	8
Reservoir	21.5	4.4	8.0	0.63	0.00	0.00	0.00	0.00	130.8	27.9	2.75	0.53	0.18	0.10	12
Distribution	23.3	3.8	8.4	0.30	0.00	0.00	0.00	0.00	158.8	15.2	--	--	--	--	8
Dixmont TPE	21.5	3.0	7.5	0.17	0.52	0.39	0.62	0.45	159	28.9	--	--	--	--	5
Reservoir	20.2	4.5	8.1	0.24	0.00	0.00	0.02	0.06	112.4	15.2	4.41	1.33	0.29	0.62	12
Distribution	22.0	3.3	7.6	0.16	0.16	0.13	0.21	0.12	121	3.6	--	--	--	--	7
Sewickley TPE	15.5	1.3	8.0	0.52	0.87	0.53	0.99	0.61	133.8	41.6	--	--	--	--	7
Reservoir No.3	15.9	2.1	7.8	0.23	0.27	0.38	0.29	0.44	124.1	13.3	6.05	1.41	0.36	0.47	12
Reservoir No.4	20.9	4.3	8.1	0.13	0.01	0.03	0.01	0.03	125.1	11.8	5.59	1.01	0.45	0.57	12
Low Pressure Distribution	17.0	2.0	7.8	0.42	0.17	0.21	0.10	0.17	*	--	--	--	--	--	3
High Pressure Distribution	21.4	1.2	7.7	0.36	0.13	0.19	0.10	0.14	176	48.1	--	--	--	--	4

N - sample size
* - one measurement only - 128

TABLE 3. ARITHMETIC MEANS ($\bar{X}$) AND STANDARD DEVIATIONS (SD) OF CHEMICAL AND PHYSICAL PARAMETERS-1978 DATA

	Temperature (°C)		pH		Hardness $CaCO_3$ mg l^{-1}		Free Cl mg l^{-1}		Total Cl mg l^{-1}		
	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	$\bar{X}$	S.D.	N
Springdale TPE	15.1	2.4	7.74	0.20	18	17.0	0.38	0.14	0.52	0.15	17
Springdale Reservoir	20.2	3.2	7.99	0.28	25	15.0	0.05	0.09	0.11	0.16	15
Springdale Distribution	22.1	2.4	7.68	0.10	20	16.7	0.36	0.73	0.46	0.72	17
McKeesport TPE	22.5	2.3	7.86	0.26	124	17.5	0.86	0.60	0.96	0.60	17
McKeesport Reservoir	22.8	2.0	7.61	0.22	133	20.0	0.06	0.11	0.09	0.11	17
McKeesport Distribution	22.8	2.3	7.94	0.26	129	15.8	0.67	0.56	0.73	0.57	16
Pittsburgh TPE	24.9	1.5	7.55	0.33	163	11.2	0.29	0.12	0.31	0.13	12
Pittsburgh Reservoir	24.7	2.0	7.70	0.20	168	13.0	0.28	0.86	0.28	0.86	12
Pittsburgh Distribution	25.3	1.9	7.64	0.14	169	10.2	0.09	0.12	0.13	0.14	9
Dixmont TPE	23.1	2.4	7.35	0.48	149	17.4	1.04	1.15	1.16	1.09	6
Dixmont Reservoir	23.6	1.1	7.65	0.35	142	17.1	0	0	0.02	0.42	15
Dixmont Distribution	24.8	1.8	7.57	0.45	139	17.4	0.04	0.09	0.07	0.10	15
Sewickley TPE	16.2	1.4	7.76	0.37	129	32.0	0.97	0.78	1.52	1.03	15
Sewickley Reservoir No. 3 (open)	16.5	0.5	7.79	0.20	122	9.5	0.08	0.08	0.16	0.11	6
Sewickley Reservoir No. 3 (covered)	19.7	1.5	7.63	0.30	128	11.0	0.27	0.19	0.41	0.24	8
Sewickley Reservoir No. 4 (open)	23.3	0.5	8.30	0.20	97	8.1	0	0	0	0	4
Sewickley Reservoir No. 4 (covered)	21.2	1.9	7.73	0.14	126	6.0	0.09	0.14	0.09	0.14	11
Sewickley Low Pressure distribution	19.5	0.9	7.68	0.20	122	6.0	0.13	0.17	0.18	0.18	14
Sewickley High Pressure distribution	21.3	1.0	7.70	0.26	125	11.4	0.06	0.09	0.09	0.13	15

N - sample size

Hardness

The hardness was generally stable and did not fluctuate substantially as most of the plants adjust it accordingly. Most of the values approximated 100 mg as $CaCO_3 l^{-1}$, seldom exceeding 170 mg. The only plant which does not adjust hardness substantially is the Springdale facility which produces very soft water.

Chlorine

The free- and total chlorine residuals decreased during transit from the treatment plant through the reservoir system. However, the decreases in chlorine levels were less noticeable in those distribution systems using post-chlorination, either directly in the reservoir (McKeesport), or between and after reservoirs (Sewickley). The Pittsburgh distribution system is so large that even additional chlorination is not effective in maintaining sufficient chlorine residuals in all areas.

If the recommendations for free chlorine residuals (EPA 1975) are to be adhered to, it is evident that the 0.3 mg l^{-1} minimum was rarely maintained in several of the distribution systems. The Springdale treatment plant maintained a sufficient free chlorine residual because the finished water is pumped directly into the distribution system during the day while the water stored in the reservoir is used at night when the chlorine concentration may drop to zero. McKeesport distribution system water has high free and total chlorine content due to effective post-chlorination in the reservoir.

There is no doubt that the open, finished water reservoirs permit substantial chlorine dissipation unless additional chlorination is provided (Lippy, 1978). Moreover, the decomposing leaves and litter introduced to the reservoirs from the surrouding environment contribute to the demand for chlorine.

Nutrients

The results of the chemical analysis for nitrates, ammonia, and orthophosphates performed in 1977 suggest that there were sufficient concentrations of nutrients in the reservoirs to support growth of algae up to and beyond the bloom level. In only one instance was a deficient concentration of orthophosphates observed in a reservoir. On June 15, the level of this nutrient in Dixmont reservoir (south) was essentially zero.

The continuous influx of nutrients in the reservoirs is assured by rainfall, leaves and other organic debris, and by polyphosphate treatment practiced by some treatment plants for iron control.

Standard Plate Count Bacteria

The only bacterial group examined during the 1977 sampling period was Standard Plate Count bacteria incubated at 35°C for 48 hours. Concentrations of SPC bacteria were usually very low in the finished water, greater in the distribution systems and greatest in the reservoirs (Table 4). The exception was McKeesport distribution system where high free residual chlorine was

TABLE 4. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF STANDARD PLATE BACTERIA (COLONIES ml^{-1}) INCUBATED AT 35°C-1977 DATA

Locality	N	Standard Plate Count Bacteria Colonies ml^{-1} $\bar{x}$	C.I.
Springdale TPE	8	1.5	0.9-2.5
Reservoir	8	850	300-2400
Distribution	8	7.1	2-26
McKeesport TPE	8	3.6	1.5-8.4
Reservoir	8	82	15-450
Distribution	8	2.9	1.2-6.6
Pittsburgh TPE	6	5.6	0.3-96
Reservoir	8	590	120-2900
Distribution	8	350	66-1900
Dixmont TPE	6	33	0.5-2300
Reservoir	8	140	31-668
Distribution	8	660	220-2000
Sewickley TPE	8	2.1	1.3-3.3
Reservoir No. 3	8	10	1.4-70
Reservoir No. 4	8	2500	1000-5800
Low Pressure Distribution	4	1	0.5-2
High Pressure Distribution	5	209	17-2500

All statistical calculations performed on log transformed data. Treat observations of zero cells (colonies) per ml as equal to one cell (colony) per ml.
$\bar{x}$ - Geometric mean; C.I.-Confidence interval; N - Sample size.

maintained, resulting in reduced bacteria count. The SPC bacteria were also very low in the Sewickley Low Pressure distribution system due to additional chlorination of reservoir water. The greatest bacterial counts were recorded in Sewickley reservoir No. 4 followed by the Springdale reservoir. Generally, the density of bacteria was lower in the distribution systems than in the reservoirs. Only Dixmont tap water revealed a greater bacterial concentration than the reservoir water. Relatively low density of bacteria were recorded in the McKeesport reservoir and Sewickley reservoir No. 3.

The SPC procedure performed in 1978 samples included incubation at 20°C and 35°C for 2 and 8 days (Table 5 and 6). Gram-negative bacteria considered to be a major source of endotoxins were counted for both temperatures and incubation periods (Table 7 and 8). There was a substantial increase in bacterial counts after eight days at both temperatures at almost all localities. The only exception was Sewickley Reservoir No. 3 (open). In this reservoir there was no noticeable increase between bacteria incubated for 2 days and 3 days at 35°C, while the SPC colonies growing at 20°C registered a substantial increase. This may have been caused by the predominance of psychrophilic bacteria growing readily at lower temperatures prevailing in the reservoir ($\bar{X}$ = 16.5°C). This relationship was not so obvious after the reservoir was covered, but the average temperature then increased to 19.7°C. This observed temperature selectivity is significant because most of the pathogenic bacteria are mesophilic, growing best at 35°C.

In the remaining reservoirs the increase from SPC bacteria incubated for 2 days to counts obtained after 8 days was substantial and especially conspicuous in Springdale, Dixmont, and Sewickley No. 4 Reservoirs. This corresponds well with elevated endotoxin concentrations at the same localities. Increase in bacterial counts from 2 days to 8 days incubation in finished water (TPE) were relatively small, while the SPC bacteria isolated from the distribution systems have shown a substantial increase in numbers.

The results of the qualitative analysis performed on SPC bacteria indicated that most of the colonies were formed by gram-negative organisms. The most common group were Flavobacteria with occasional occurrence of Pseudomonas and Serratia. Some Flavobacteria may cause meningitis, whereas some of the Pseudomonas and Serratia species are causative agents of waterborne skin infections and gastroenteritis (Finegold et al., 1978; Hoadley, 1977).

The gram-negative bacteria were found to be at elevated levels in several reservoirs namely at Dixmont and Sewickley No. 4, which may have contributed to increases in endotoxin concentration in these two reservoirs. From distribution systems the greatest gram-negative bacterial counts were recorded in Dixmont, evidently as the result of reservoir contamination. The results (Tables 5-8) indicate that SPC bacteria technique as recommended in Standard Methods (1975), reflects only the fraction of bacterial population which develops within 2 days. The sensitivity of this method can be greatly increased by extended incubation (8 days).

A new medium for the enumeration and subculture of bacteria from drinking waters has been investigated recently by Reasoner and Geldreich

TABLE 5. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF STANDARD PLATE COUNT BACTERIA (COLONIES ml^{-1}) INCUBATED AT~20°C - 1978 DATA

Locality	N	2 days incubation $\bar{x}$	2 days incubation C.I.	8 days incubation $\bar{x}$	8 days incubation C.I.
Springdale TPE	14	4.7	1.9-12	49	18-130
Springdale Reservoir	14	240	49-1100	1800	710-4600
Springdale Distribution	13	7.2	2.0-26	130	39-460
McKeesport TPE	12	3.5	1.5-8.6	11	3.4-37
McKeesport Reservoir	14	24	4.1-140	180	40-770
McKeesport Distribution	10	8.8	1.7-46	55	10-310
Pittsburgh TPE	12	4.0	1.2-14	20	6.0-68
Pittsburgh Reservoir	5	19	0.63-590	440	42-4700
Pittsburgh Distribution	7	10	2.2-51	560	86-3700
Dixmont TPE	6	1.3	0.87-1.8	41	6.6-250
Dixmont Reservoir	10	700	160-3100	2200	770-6400
Dixmont Distribution	11	250	35-1800	2900	970-8500
Sewickley TPE	13	3.4	1.0-11	31	14-70
Sewickley Reservoir No. 3 (open)	4	240	2.2-26000	450	3.3-61000
Sewickley Reservoir No. 3 (covered)	7	190	17-2200	790	410-1500
Sewickley Reservoir No. 4 (open)	2	350	$0-2.5x10^9$	7300	$4.8x1.1x10^7$
Sewickley Reservoir No. 4 (covered)	6	480	18-13000	2200	600-8400
Sewickley Low Pressure	10	17	2.2-130	130	18-1000
Sewickley High Pressure	12	5.5	1.6-19	560	210-1500

$\bar{x}$ - Geometric mean
C.I. - Confidence interval
N - Number of samples

TABLE 6. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF STANDARD PLATE COUNT BACTERIA (COLONIES ml-1) INCUBATED AT 35°C - 1978 DATA

Locality	N	2 days incubation $\bar{x}$	2 days incubation C.I.	8 days incubation $\bar{x}$	8 days incubation C.I.
Springdale TPE	14	3.6	2.0-6.4	21	7.7-55
Springdale Reservoir	14	200	40-1000	1100	490-2300
Springdale Distribution	13	26	7.3-92	130	30-580
McKeesport TPE	12	12	4.6-31	13	4.8-36
McKeesport Reservoir	14	100	24-410	310	11-890
McKeesport Distribution	10	21	5.5-82	19	3.8-96
Pittsburgh TPE	12	16	4.7-57	14	3.6-56
Pittsburgh Reservoir	5	110	3.2-3400	300	4.5-19000
Pittsburgh Distribution	7	33	2.3-460	250	21-2900
Dixmont TPE	6	2.7	0.49-14	7.5	1.0-55
Dixmont Reservoir	10	650	97-4400	1400	340-6100
Dixmont Distribution	11	530	110-2600	1900	1000-3600
Sewickley TPE	13	3.4	1.3-9.1	10	3.5-29
Sewickley Reservoir No. 3 (open)	4	650	20-21000	630	24-17000
Sewickley Reservoir No. 3 (covered)	7	100	7.6-1400	230	39-1400
Sewickley Reservoir No. 4 (open)	2	2300	160-34000	4400	1800-10000
Sewickley Reservoir No. 4 (covered)	6	260	13-4900	1100	500-2500
Sewickley Low Pressure	10	7.7	1.5-39	110	19-690
Sewickley High Pressure	12	28	5.3-150	160	34-750

$\bar{x}$ - Geometric mean
C.I. - Confidence interval
N - Number of Samples

TABLE 7. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF GRAM-NEGATIVE (COLONIES ml^{-1}) INCUBATED AT 20°C - 1978 DATA

Locality	N	2 days incubation $\bar{x}$	2 days incubation C.I	8 days incubation $\bar{x}$	8 days incubation C.I.
Springdale TPE	14	1.9	0.95-3.8	25	8.8-73
Reservoir	14	83	14-500	980	370-2600
Distribution	13	3.2	1.0-10	20	4.1-100
McKeesport TPE	12	1.4	0.84-2.4	5.8	1.8-18
Reservoir	14	8.8	1.4-55	77	15-410
Distribution	10	2.0	0.64-6.4	21	4.3-100
Pittsburgh TPE	12	1.9	0.71-4.9	9.4	2.2-40
Reservoir	5	5.4	0.53-55	210	28-1600
Distribution	7	2.9	0.83-10	290	48-1700
Dixmont TPE	6	1.0	1.0-1.0	13	1.0-180
Reservoir	10	46	4.3-480	2500	890-6900
Distribution	11	21	2.2-200	2000	770-5400
Sewickley TPE	13	2.2	0.77-6.0	21	9.3-47
Reservoir No. 3 (open)	4	61	0.03-120	260	1.2-57000
Reservoir No. 3 (covered)	7	78	6.8-900	520	260-1000
Reservoir No. 4 (open)	2	7.8	$0-1.6 \times 10^{12}$	4800	450-51000
Reservoir No. 4 (covered)	6	410	31-5400	1700	430-6700
Low Pressure System	10	5.1	1.1-25	90	14-560
High Pressure System	12	2.4	1.0-5.9	170	28-1000

$\bar{x}$ - Geometric mean
C.I. - Confidence interval
N - Number of samples

TABLE 8. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF GRAM-NEGATIVE BACTERIA (COLONIES ml^{-1}) INCUBATED AT 35°C-1978 DATA

Locality	N	2 Days Incubation $\bar{x}$	2 Days Incubation C.I.	8 Days Incubation $\bar{x}$	8 Days Incubation C.I.
Springdale TPE	14	1.4	0.91-2.2	11	3.8-34
Reservoir	14	67	9.3-480	270	68-1100
Distribution	13	3.6	1.0-13	13	3.1-52
McKeesport TPE	12	2.3	0.97-5.2	4.5	1.8-11
Reservoir	14	43	8.9-200	140	40-530
Distribution	10	2.5	0.87-7.1	8.9	2.3-34
Pittsburgh TPE	12	5.7	1.6-20	7.0	1.9-27
Reservoir	5	78	3.2-1900	120	2.2-6800
Distribution	7	23	1.7-310	110	14-870
Dixmont TPE	6	1.1	0.83-1.5	5.0	0.62-40
Reservoir	10	170	17-1800	740	160-3500
Distribution	11	180	27-1200	660	110-3900
Sewickley TPE	13	2.2	0.88-5.4	6.1	2.5-15
Reservoir No. 3 (open)	4	28	0.03-28000	390	13-11000
Reservoir No. 3 (covered)	7	63	4.2-930	170	33-890
Reservoir No. 4 (open)	2	16	$0-38 \times 10^{16}$	3000	830-11000
Reservoir No. 4 (covered)	6	180	15-2100	720	290-1800
Low Pressure System	10	2.5	0.63-9.9	43	7.8-230
High Pressure System	12	7.4	1.3-43	80	21-270

$\bar{x}$ - Geometric mean
C.I. - Confidence interval
N - Number of samples

(1979). The investigators showed that samples incubated on SPC media at 35°C for 48 hours resulted in 30 colonies ml^{-1}, whereas, delayed incubation (7 days) increased the number of bacteria to 73 colonies ml^{-1}. The new medium provided 1,200 colonies ml^{-1} after 48 hours, 2,300 colonies ml^{-1} after 72 hours, and 4,300 colonies ml^{-1} after 7 days of incubation. Herman (1976) has shown that the predominant survivors in chlorinated domestic water supplies are "the slow growing pigmented water bacteria" also often classified as Flavobacteria.

According to our observations, which correspond with those of Herman (1976), the extended incubation is especially effective for detection of inadequate water treatment. This was demonstrated by the fact that the elevated counts observed after delayed incubation were especially conspicuous in open water reservoirs which are frequently contaminated by bacteria. The evidence that several groups of slow-growing bacteria may include potential pathogens suggests that especially debilitated or immunosuppressed patients in hospitals, other health care facilities, and/or in the general population are most likely to be affected by these species.

Coliform Bacteria

Total and fecal coliform bacteria were determined during the 1978 sampling period. Both groups were absent in finished water samples and rare in distribution system samples. The Springdale distribution system was devoid of any coliform contamination whereas the remaining distribution systems were only occasionally contaminated by total coliform bacteria. Occasional positive samples are probably due to contamination that occurred in the reservoirs. The greatest total coliform counts were recorded in Sewickley reservoirs after covers were installed with lesser counts noted in Pittsburgh and Dixmont reservoirs. Fecal coliforms were found once in the Pittsburgh reservoir and on three occasions in Sewickley Reservoir No. 4 after covering.

These results show that total and fecal coliform bacteria were rarely found in open reservoirs. Therefore, their value as water quality indicators under these circumstances is limited. A combination of SPC bacteria, algae, and especially endotoxin analysis may provide a better measure of the biological contamination of open, finished water storage facilities.

The uncovered, Sewickley Reservoir samples were devoid of coliforms. However, these bacteria appeared in the water samples almost immediately after the covers were installed. The synthetic rubber lining on the reservoir walls was treated with large doses of chlorine which evidently eliminated possible contamination from this source. The only "weak spots" in the covers are the hatches (three in each reservoir) which served as sampling points. They are located on floats, slightly elevated, and equipped with zippers. The central section of each hatch forms a basin in which rainwater accumulates. This water is usually full of insect and plant remains and quite often racoon tracks were observed on the bottom of a hatch filled with water. It could be expected that when water in this "container" overflows and drips through the zipper into the reservoir it contaminates the reservoir sampling location.

A special sampling trip was undertaken on October 25, 1978, to investigate whether contamination was associated with the hatch covers. The samples were collected from the accumulated rainwater, condensate inside of the hatch, and reservoir water. Both Sewickley Reservoirs (No. 3 and 4) were sampled at the same time. The hatch on Reservoir No. 4 was almost empty, while the hatch on Reservoir No. 3 was full of rainwater. The rainwater and the condensate collected from the inside of the hatch were contaminated by total coliforms with high count recorded from Reservoir No. 3 hatch water and condensate. The water in Reservoir No. 3 was contaminated by 22 total coliforms ml^{-1}, while the samples collected in Reservoir No. 4 contained only 4 colonies ml^{-1}. These results suggest that the condensate inside of the hatch is contaminated by rainwater dripping through the zipper which in turn contaminates the drinking water stored in the reservoir (Table 9) and may result in positive samples collected from the distribution system.

TABLE 9. TOTAL COLIFORM BACTERIA IN RAINWATER FROM HATCHES, CONDENSATED WATER AND RESERVOIR WATER - SEWICKLEY NO. 3 and 4, 25 OCTOBER 1978

Locality	Total Coliforms (Colonies/100 ml)
Reservoir No. 3	
Hatch rainwater	100
Hatch condensate	1,400
Surface	22
Bottom	0
Reservoir No. 4	
Hatch rainwater[1]	100
Hatch condensate	22
Surface	4
Bottom	0

[1] This hatch was almost empty.

Phytoplankton

In 1977, the highest concentrations of autotrophic organisms were found in the Springdale Reservoir followed by Dixmont and Sewickley No. 4 Reservoirs. Low concentrations of autotrophic organisms were recorded in McKeesport and Sewickley No. 3 Reservoirs. The distribution systems were occasionally contaminated by large cellular concentrations of algae and Cyanobacteria, especially the Dixmont and Springdale distribution systems (Table 10). The dominant species in all facilities was Chlorella vulgaris, an ubiquitous green alga, evidently less sensitive to chlorine and copper sulphate which are used for reservoir treatment, than any other phytoplankton organism. From the group of Cyanobacteria the most commonly occurring species was a brown strain of Schizothrix calcicola, which was often encountered in samples from McKeesport, Sewickley No. 4 and Pittsburgh (Highland Park I) reservoirs (Table 11). Sixty-seven percent of samples collected at McKeesport reservoir were contaminated by this species while in Sewickley No. 4 and Pittsburgh reservoirs it occurred in 56% and 42% of the samples, respectively.

In 1978 autotrophic organisms were also a common occurrence in open finished water reservoirs with greatest cellular counts recorded from Springdale reservoir followed by Sewickley No. 4 (uncovered), Dixmont, Pittsburgh, McKeesport and Sewickley No. 3 (uncovered) reservoirs (Table 12). In the Springdale, Dixmont, and Pittsburgh systems, algal counts in the reservoirs were ten to twenty times as great as those in the finished waters. Sewickley reservoir No. 4 was a major source of algae for the Sewickley High Pressure Distribution System before a reservoir cover was placed on it. Low algal counts found in Sewickley Reservoir No. 3 resulted from the relatively high chlorine content and low temperatures prevailing in this reservoir. Before it was covered, Sewickley Reservoir No. 3 algal and Cyanobacterial counts revealed a geometric mean of 310 cells per ml. After it was covered the geometric mean dropped to 6.3 total autotrophic organisms per ml.

The autotrophic organisms cellular densities recorded from finished waters were usually low and originated from growth on filter walls and other facilities inside of treatment plants, and penetration of algae through the filtration system (McKeesport). All of the finished water studied had algal counts usually less than 300 total algae per ml. The geometric means of the algal and Cyanobacterial counts from the McKeesport and Dixmont finished waters were higher than those from the other systems because high concentrations of autotrophic organisms reaching tens-of-thousands of cells per ml were recorded in these systems on several occasions. Both the McKeesport and Dixmont systems use river water that may be the source of contamination by algae. Of prime interest is that the heterotrophic bacteria counts in the McKeesport and Dixmont finished waters, even on those days with high algal counts, were not any higher than the bacterial counts in the other finished waters. This indicates that the disinfection was effective in both systems. It is, therefore, possible the most of the algae counted in these samples were actually dead cells.

The major source of autotrophic organisms in the distribution systems are open water reservoirs. After the Sewickley reservoirs were covered,

TABLE 10. GEOMETRIC MEANS AND 95% CONFIDENCE INTERVALS OF PHYTOPLANKTON - 1977 DATA

Locality	Cyanobacteria cells ml^{-1} N	$\bar{x}$	C.I.	Total Phytoplankton cells ml^{-1} N	$\bar{x}$	C.I.
Springdale TPE	--	--	--	--	--	--
Reservoir	11	3.8	0.7- 19	11	260,000	200,000-350,000
Distribution	9	7	0.7- 69	9	4,000	2,300- 6,900
McKeesport TPE	--	--	--	--	--	--
Reservoir	12	47	6.6-330	12	900	350- 2,400
Distribution	9	15	1.2-190	19	1,400	400- 4,500
Pittsburgh TPE	--	--	--	--	--	--
Reservoir	12	6.9	1.5- 33	12	3,000	1,400- 6,800
Distribution	9	5	0.7- 33	9	1,900	750- 4,800
Dixmont TPE	--	--	--	--	--	--
Reservoir	10	3.3	0.8- 21	10	48,000	20,000-120,000
Distribution	8	1.9	0.4- 8.2	8	12,000	2,100- 66,000
Sewickley TPE	--	--	--	--	--	--
Reservoir No. 3	10	2.8	0.8- 9.5	10	220	80- 620
Reservoir No. 4	9	14	1.6-126	9	3,200	420- 24,000
Low Pressure distribution	4	0	0	4	240	120- 520
High Pressure distribution	4	0	0	4	920	130- 6,700

All statistical calculations performed on log transformed data treat observations of zero cells (colonies) per ml as equal to one cell (colony) per ml.

$\bar{x}$ - Geometric mean
C.I. - Confidence interval
N - Sample size

TABLE 11. PERCENT COMPOSITION OF CHLORELLA, CYANOBACTERIA AND SCHIZOTHRIX CALCICOLA IN 1977 SAMPLES

	Chlorella % of total Algae	Cyanobacteria % of total Algae	S. calcicola % of Cyano-bacteria	S. calcicola frequency occurrence in samples (%)
Springdale TPE	--	--	--	--
Reservoir	99.5	0.1	99.3	18
Distribution	97.2	1.0	0	0
McKeesport TPE	--	--	--	--
Reservoir	64.3	21.8	98.2	67
Distribution	72.3	6.6	55.8	44
Pittsburgh TPE	--	--	--	--
Reservoir	96.7	1.0	100	42
Distribution	95.3	1.5	0	0
Dixmont TPE	--	--	--	--
Reservoir	98.7	0.2	100	20
Distribution	99.3	0.1	100	13
Sewickley TPE	--	--	--	--
Reservoir #3	95.2	2.2	100	30
Reservoir #4	88.9	0.8	100	56
Low Pressure	94.6	0	0	0
High Pressure	94.8	0	0	0

TABLE 12. 95% CONFIDENCE INTERVALS AND GEOMETRIC MEANS OF PHYTOPLANKTON COUNTS-1978 DATA

	Total Phytoplankton cells ml^{-1}			Cyanobacteria cells ml^{-1}	
Sampling Location	N	C.I.	$\bar{x}$	C.I.	$\bar{x}$
Springdale TPE	17	15-130	44	0.86-2.8	1.6
Springdale Reservoir	15	35000-150000	73000	2.9-34	9.9
Springdale Distribution	17	190-2500	690	0.84-2.6	1.5
McKeesport TPE	17	26-500	110	1.3-10	3.5
McKeesport Reservoir	17	190-1700	560	28-290	90
McKeesport Distribution	16	33-240	88	0.89-4.4	2.0
Pittsburgh TPE	11	7.3-100	27	0.69-2.7	1.4
Pittsburgh Reservoir	12	1100-9100	3200	120-5000	770
Pittsburgh Distribution	9	120-2100	490	7.0-140	31
Dixmont TPE	6	6-4400	160	0.55-3.9	1.5
Dixmont Reservoir	15	920-1300	[illegible]	0.95-1.2	1.1
Dixmont Distribution	15	610-9000	2300	0.76-16	3.5
Sewickley TPE	15	7.1-140	31	0.76-3.5	1.6
Sewickley Reservoir #3 (open)	6	25-3900	310	1.8-120	14
Sewickley Reservoir #3 (covered)	7	1.6-25	6.3	0.75-2.0	1.2
Sewickley Reservoir #4 (open)	4	4300-290000	36000	130-6400	900
Sewickley Reservoir #4 (covered)	8	3.5-130	21	0.83-1.6	1.2
Sewickley Low Pressure System	14	28-150	65	1.3-14	4.3
Sewickley High Pressure System	15	25-690	130	1.1-12	3.7

N = Number of samples
C.I. = Confidence interval
$\bar{x}$ = Geometric mean

the algal counts decreased gradually in the reservoirs as well as in both distribution systems. However, some algae survived for an extended period, but it was not possible to determine visually whether these organisms were dead or alive.

In 1978, the most common species found in the drinking water was again C. vulgaris. However, S. calcicola was a significant component of the phytoplankton in Pittsburgh and McKeesport reservoirs representing 74.5% and 18% of total phytoplankton counts respectively. S. calcicola was also common in the Pittsburgh distribution system occurring in 89% of the samples and constituting 10% of total phytoplankton population. The same species was also frequently encountered in low numbers in samples from Sewickley's low pressure distribution system (in 80% of samples - Table 13). The two study results indicate that McKeesport reservoir contains a permanent population of S. calcicola while its occurrence in the Pittsburgh system is sporadic and varies from one year to the other.

It is important to note that rarely was S. calcicola the dominant species in any reservoir as it was during the Sewickley outbreak in 1975. The one exception is the Pittsburgh reservoir in 1978 when S. calcicola was the leading species and its population reached 5,000 cells ml^{-1}. In Sewickley during the outbreak the concentration was almost 100 times that number. It is not yet clear what occurred at Sewickley in 1975 that permitted S. calcicola to suppress C. vulgaris, which is normally the dominant phytoplankton organism in open, finished water reservoirs.

Endotoxin

Endotoxin concentrations varied from nondetectable levels in some finished water samples (plant effluents) to as high as 250 ng ml^{-1} in some of the open reservoirs. In 1977 the exact concentrations of endotoxins in the water were not determined and only differences in order of magnitude were recorded. However, it is significant that none of the assays in the 1977 study indicated levels of 2,500 ng ml^{-1} such as those reported from the Sewickley water supply shortly after the 1975 epidemic of gastroenteritis. Cursory examination of Table 14 indicates that in general, endotoxin concentrations were at lowest levels in plant effluents followed by a substantial increase in the reservoirs and/or distribution systems.

Great effort was expended to detect actual endotoxin concentrations in the 1978 samples. Table 15 shows the geometric means and 95% confidence limits of endotoxin concentrations of the respective sample locations. Endotoxin values varied from nondetectable levels in some finished water samples (plant effluents) to as high as 630 ng ml^{-1}, in Sewickley reservoir No. 4.

The McKeesport system exhibited the highest levels of endotoxin in the plant effluent which could be explained by the use of river water as the source of raw water and by the absence of prechlorination. All the remaining systems displayed generally low concentrations ranging from zero to 6.3 ng ml^{-1} in the plant effluent which was due to improved treatment techniques or due to the use of groundwater as raw water source.

TABLE 13. PERCENT COMPOSITION OF CHLORELLA, CYANOBACTERIA AND SCHIZOTHRIX CALCICOLA IN 1978 SAMPLES

	Chlorella % of total Algae	Cyanobacteria % of total Algae	S. calcicola % of Cyano-bacteria	S. calcicola frequency occurrence in samples (%)
Springdale TPE	80.5	3.5	94.8	17
Reservoir	98.9	0.1	100	60
Distribution	98.6	0.1	100	12
McKeesport TPE	96.9	1.0	99.4	41
Reservoir	81.0	18.0	100	94
Distribution	89.1	4.3	100	31
Pittsburgh TPE	93.2	3.4	100	9
Reservoir	25.0	74.5	100	92
Distribution	85.5	10.2	99.5	89
Dixmont TPE	98.3	0.01	0	0
Reservoir	99.7	0.001	100	7
Distribution	85.5	13.6	100	33
Sewickley TPE	22.8	2.6	6.8	7
Reservoir #3 (open)	54.3	6.0	100	83
Reservoir #3 (covered)	91.8	4.1	100	14
Reservoir #4 (open)	96.3	1.9	100	100
Reservoir #4 (covered)	98.4	0.4	100	13
Low Pressure (open)	49.6	31.8	100	80
Low Pressure (covered)	77.5	19.1	100	22
High Pressure (open)	87.1	4.7	100	50
High Pressure (covered)	97.0	2.6	100	22

TABLE 14. STATISTICAL ANALYSIS OF ENDOTOXIN CONCENTRATIONS-1977 DATA

System	Endotoxin ($ng\ ml^{-1}$) $\bar{x}$	Mode	Range	N
Springdale TPE	2.5	2.5	2.5	3
Reservoir	25	25	25-250	6
Distribution	7.9	25	2.5-25	3
McKeesport TPE	7.9	25	2.5-25	4
Reservoir	4.8	2.5	2.5-25	7
Distribution	12	25	2.5-25	3
Pittsburgh TPE	2	2.5	0-2.5	4
Reservoir	9.3	25	2.5-25	7
Distribution	25	25	25	3
Dixmont TPE	5.4	2.5	2.5-25	4
Reservoir	120	250	25-250	6
Distribution	25	25	25	4
Sewickley TPE	2.1	2.5	0-2.5	5
Reservoir #3	2.5	2.5	25	6
Reservoir #4	54	250	25-250	6
Distribution #1	1.8	2.5	2.5	2
Distribution #2	2.5	2.5	0-2.5	3

$\bar{x}$ = Geometric mean

TABLE 15. 95% CONFIDENCE INTERVALS AND GEOMETRIC MEANS OF ENDOTOXINS-1978 DATA

Sampling Location	Endotoxin (ng ml^{-1}) N	C.I.	$\bar{x}$
Springdale TPE	17	0.61-1.3	0.90
Springdale Reservoir	15	4.5-22	9.9
Springdale Distribution	17	0.87-2.0	1.3
McKeesport TPE	17	0.81-3.6	1.7
McKeesport Reservoir	17	0.72-4.3	1.8
McKeesport Distribution	16	0.98-5.0	2.2
Pittsburgh TPE	12	0.32-1.0	0.57
Pittsburgh Reservoir	12	2.1-9.8	4.6
Pittsburgh Distribution	9	0.31-2.0	0.78
Dixmont TPE	6	0.43-1.5	0.80
Dixmont Reservoir	15	2.1-10	4.6
Dixmont Distribution	15	1.8-5.4	3.1
Sewickley TPE	15	0.37-1.5	0.73
Sewickley Reservoir No. 3 (open)	6	0.52-3.1	1.3
Sewickley Reservoir No. 3 (covered)	8	0.31-1.3	0.63
Sewickley Reservoir No. 4 (open)	8	4.9-3200	130
Sewickley Reservoir No. 4 (covered)	11	2.7-16	6.5
Sewickley Low Pressure System	14	0.55-2.9	1.3
Sewickley High Pressure System	15	1.1-6.7	2.7

N = Number of samples

C.I. = Confidence interval

$\bar{x}$ = Geometric mean

By these criteria the Pittsburgh system represents an exception. The treatment plant utilizes water directly from the Allegheny River; yet the plant effluent exhibited a geometric mean of only 0.57 ng ml^{-1} which is substantially lower than endotoxin concentration in the water produced at the other treatment plants. A possible explanation for this occurrence is that Pittsburgh practices prechlorination of raw water which may be responsible for the elimination of most endotoxin.

With the exception of McKeesport reservoir and Sewickley reservoir No. 3 generally the greatest endotoxin levels were recorded in the open reservoirs in comparison to plant effluent and distribution systems. The geometric means of the endotoxin concentrations (in nannograms) detected in reservoirs were 1.06 - 100 times higher than geometric means of LPS detected in finished waters. During this sampling period (Table 15), the greatest endotoxin concentrations occurred in small reservoirs-Sewickley No. 4 and Springdale. The McKeesport Reservoir displayed almost identical endotoxin levels when compared with the water produced at the plant which can be attributed to the booster chlorination provided to the water in the reservoir. Sewickley Reservoir No. 3 (open) displayed also very low endotoxin concentrations, which were accompanied by low algal counts. The water temperature averaged only 16.5ºC and was probably responsible for this phenomenon.

In the 1978 samples from the distribution systems the endotoxin concentrations were again higher than in the plant effluents but slightly lower than the values obtained from the open reservoirs. The increase in endotoxin levels could be attributed to the bacterial contamination in the distribution systems, decomposition of algae and bacteria originating in open reservoirs, and decreasing chlorine levels in the mains.

In August 1975, Sewickley Reservoir No. 3 and 4 were covered with permanent plastic covers, four weeks and six weeks, respectively, after our sampling program was initiated. This action had a profound effect on all contaminants in the reservoirs and resulted in substantial reduction of endotoxin concentrations (Table 15).

A review of the results of the chemical and endotoxin analyses shows that the highest endotoxin concentrations occur in waters devoid of chlorine. Sewickley Reservoirs No. 3 and 4 demonstrate this concept well. As chlorine concentrations decrease in the Sewickley reservoirs, endotoxin concentrations increase. A simple linear regression analysis of endotoxin concentrations versus free and total chlorine yielded a highly significant relationship between the three variables when a semi-logarithmic formula was used and the logarithms of the endotoxin concentrations were designated as dependent variables (Table 16). When a simple linear regression analysis was performed only on the endotoxin and chlorine data obtained from the reservoir samples, the relationship between endotoxin and total chlorine was significant at a 0.012 level. No significant relationship, however, was found between reservoir endotoxin concentrations and free chlorine. It is expected that endotoxin and chlorine directly affect one another, but there are many other constituents such as iron, manganese, sulfite, phenol, ammonia, and algae that exert a chlorine demand. Therefore, what appears to be a causal relationship between endotoxin degradation and chlorine content may in fact be only an

association between conditions which affect all these factors.

TABLE 16. PEARSON'S CORRELATION COEFFICIENTS AND SIGNIFICANCE LEVELS OF SIMPLE LINEAR REGRESSION ANALYSES OF ENDOTOXIN AND CHLORINE (1978 DATA)

Source of Sample	Y	X	r	s
All data	Log LPS	Free Chlorine	-0.17784	0.01017
Reservoir data	Log LPS	Free Chlorine	-0.10240	0.21613
All data	Log LPS	Total Chlorine	-0.26938	0.00019
Reservoir data	Log LPS	Total Chlorine	-0.28716	0.01242

Y = Dependent variable
X = Independent variable
r = Correlation coefficient
s = Significance level

Simple linear regression analysis of the biological results from samples obtained from finished waters, reservoirs, and distribution systems in 1978 indicated significant relationship between all SPC groups and endotoxin. The total algae counts as correlated to endotoxin were also found to be highly significant ($P < 0.00001$, Table 17). This is to be expected as other environmental and chemical factors in the mains and treatment plants act together effectively reducing bacteria, algae, and endotoxin. The observed relationship may, therefore, reflect the combined action of many factors without revealing the origins or sources of contamination.

Conversely, the environment in the reservoirs eliminates many of those conditions present in distribution systems and the reservoirs may reveal more about the sources and origins of Limulus amoebocyte lysate (LAL) gelation than the analysis of finished water and distribution system samples. Sunlight provides energy for organisms in uncovered reservoirs, the chlorine dissipates quickly and the excessive pressure common in pumps and distribution systems is absent.

The results of the simple linear regression analysis of the reservoir samples indicated highly significant relationship between algae and endotoxin and between SPC (35°C, 8 days) bacteria and lipopolysaccharides. The significance of the other parameters (including SPC bacteria incubated for 2 days) as related to endotoxin was not as high as the relationship found for algae (Table 18, Fig. 1, 2 and 3).

Further analyses of the reservoir data has been undertaken using a multiple regression between the most important biological and chemical

TABLE 17. PEARSON'S CORRELATION COEFFICIENTS AND SIGNIFICANCE LEVELS OF SIMPLE LINEAR REGRESSION ANALYSES OF BACTERIA, PHYTOPLANKTON AND ENDOTOXIN

ALL 1978 DATA

Y	X	r	s
Log LPS	Log SPC(20°C-2 days)	0.31761	0.00054
Log LPS	Log SPC(35°C-2 days)	0.35703	0.00001
Log LPS	Log SPC(20°C-8 days)	0.35910	0.00001
Log LPS	Log SPC(35°C-8 days)	0.42028	0.00001
Log LPS	Log GNB(20°C-2 days)	0.34528	0.00025
Log LPS	Log GNB(35°C-2 days)	0.41322	0.00001
Log LPS	Log GNB(20°C-8 days)	0.36312	0.00001
Log LPS	Log GNB(35°C-8 days)	0.37286	0.00001
Log LPS	Log Phytoplankton	0.47933	0.00001

SPC = Standard Plate Counts
GNB = Gram Negative Bacterial Counts
Y = Dependent Variable
X = Independent Variable
r = Correlation Coefficient
s = Significance Level

TABLE 18. SUMMARY OF SIMPLE LINEAR REGRESSION ANALYSIS OF PHYTOPLANKTON ORGANISMS AND BACTERIA VERSUS LPS IN RESERVOIRS - 1978 DATA

Parameter	r	s	C.I.	N
Phytoplankton	0.59630	0.00001	0.37 - 0.72	58
Gram-negative 20°C - 8 days	0.32795	0.00435	0.09 - 0.53	63
Gram-negative 35°C - 8 days	0.30985	0.00636	0.07 - 0.52	64
SPC - 20°C - 2 days	0.21337	0.03807	0 - 0.43	70
SPC - 35°C - 2 days	0.21089	0.0693	0.02 - 0.46	73
SPC - 20°C - 8 days	0.36724	0.00103	0.15 - 0.56	68
SPC - 35°C - 8 days	0.40684	0.00007	0.24 - 0.62	68

r - Correlation coefficient
s - Significance level
C.I. - Confidence Interval of r
N - Number of samples

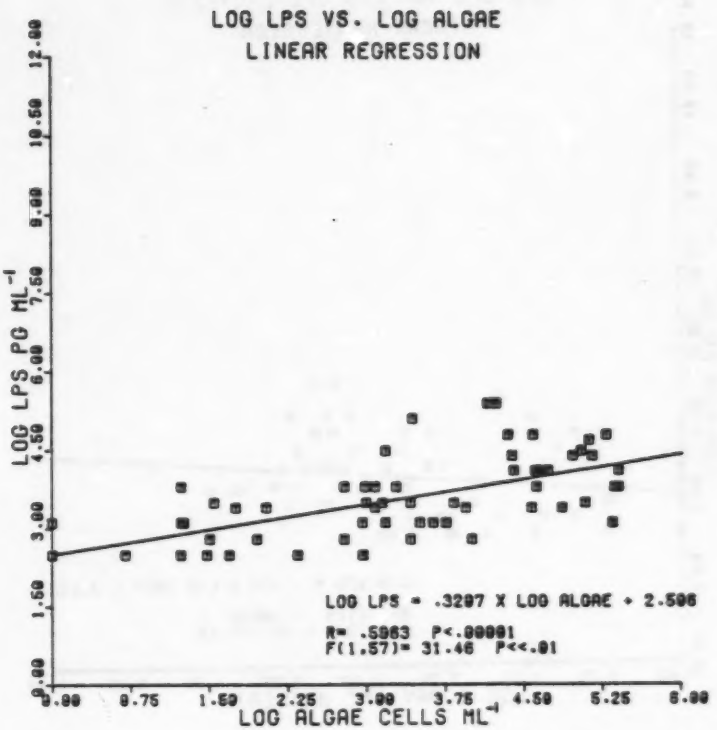

Figure 1. Correlation Between Total Phytoplankton and Endotoxin (LPS) Concentrations (in picograms-PG) in Open, Finished Water Reservoirs

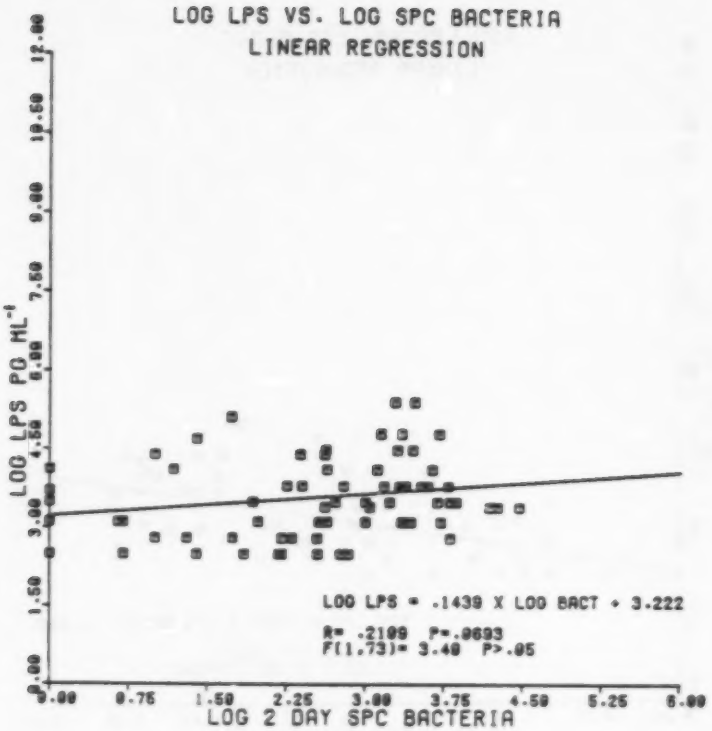
LOG LPS VS. LOG SPC BACTERIA
LINEAR REGRESSION
LOG LPS PG ML-1
LOG 2 DAY SPC BACTERIA
LOG LPS = .1439 X LOG BACT + 3.222
R= .2109 P=.0693
F(1,73)= 3.48 P>.05

Figure 2. Correlation Between SPC Bacteria (Incubated for 2 days) and LPS (in picograms-PG) in Open, Finished Water Reservoirs

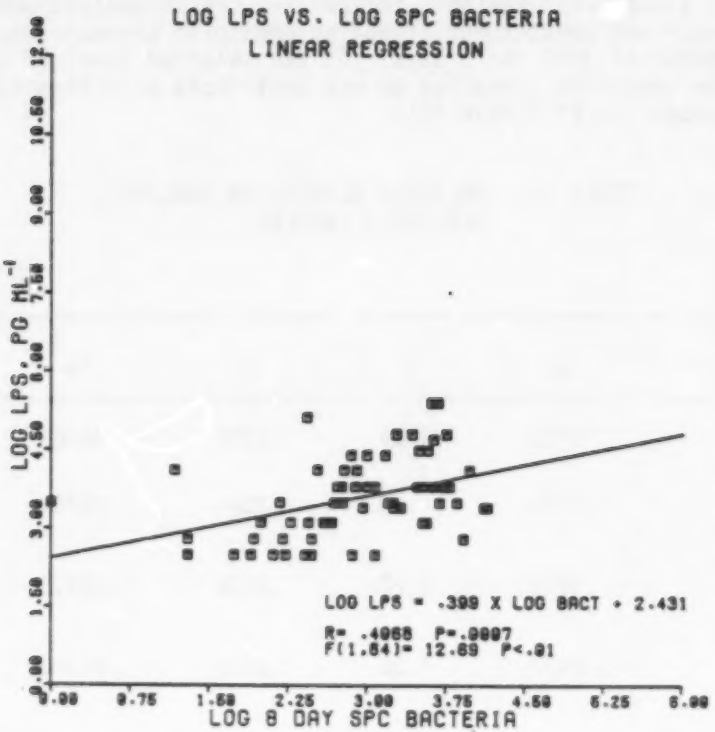
LOG LPS VS. LOG SPC BACTERIA
LINEAR REGRESSION
LOG LPS, PG ML⁻¹
LOG LPS = .399 X LOG BACT + 2.431
R= .4068 P=.0007
F(1,64)= 12.69 P<.01
LOG 8 DAY SPC BACTERIA

Figure 3. Correlation Between SPC Bacteria (Incubated for 8 days) and LPS (in picograms-PG) in Open, Finished Water Reservoirs

constituents - LPS, phytoplankton, SPC bacteria (incubated at 35°C for 2 and 8 days), free and total chlorine.

The correlation between biological contaminants and LPS in individual reservoirs was inconclusive because of the small sample size available from individual localities. However, when multiple regression analysis was applied to the pooled data from all reservoirs, the phytoplankton became again the most significant variable influencing LPS concentrations. The second most important constituent affecting endotoxin concentrations were SPC bacteria incubated at 35°C for 8 days. At the selected level of significance (s = 0.05), the remaining variables do not contribute significantly to the observed variances in LPS (Table 19).

TABLE 19. MULTIPLE REGRESSION ANALYSIS, RESERVOIR SAMPLES

	B	T	S	Δ_R^2	R^2
Phytoplankton	.2729	4.59	.0001	.35669	.35669
SPC - 35°C 2 days	-.1016	.93	.3524	.00734	.36403
SPC - 35°C 8 days	.3916	2.20	.0278	.05535	.41938
Free Chlorine	1.5492	1.26	.2076	.01783	.43721
Total Chlorine	-1.4010	1.17	.2420	.01495	.45216

Multiple R = 0.6724, R^2 = 0.4522, C = 1.8282

B = slope
T = T test on B
S = significance
R = correlation coefficient
C = intercept
Δ_R^2 = contribution to R^2
R^2 = explains variance

That algae may be responsible for LAL gelation is further supported by the results in Figure 4, 5, and 6, which show the geometric means and confidence limits of total algae and endotoxin concentrations as measured in open drinking water reservoirs. In these graphs the increases or decreases in

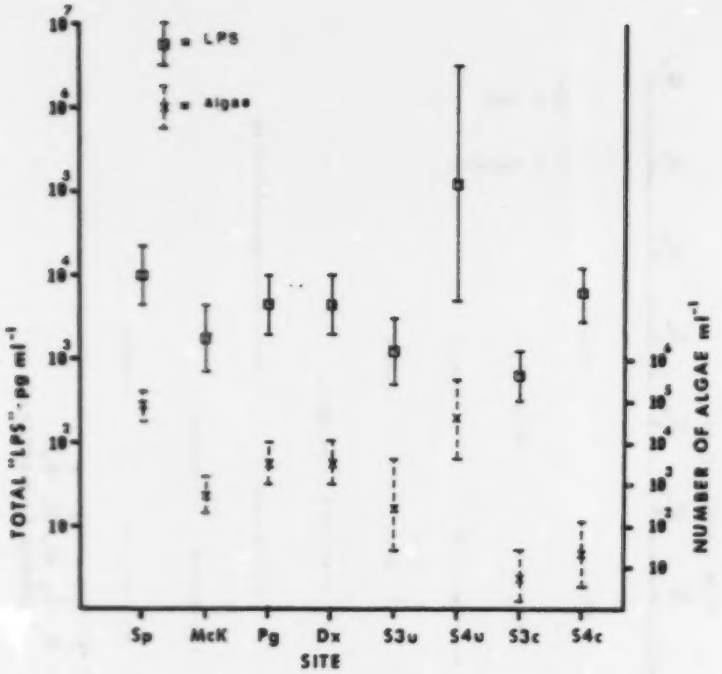

Figure 4. LPS (in picograms-pg) and Phytoplankton in Open, Finished Water Reservoirs. Vertical lines represent 95% confidence intervals with geometric means in the center.
Reservoirs localities: Sp = Springdale, McK = McKeesport.
Pg = Pittsburgh, Dx = Dixmont, S3u = Sewickley No. 3 (uncovered)
S4u = Sewickley No. 4 (uncovered), S3c = Sewickley No. 3 (covered),
S4c = Sewickley No. 4 (covered)

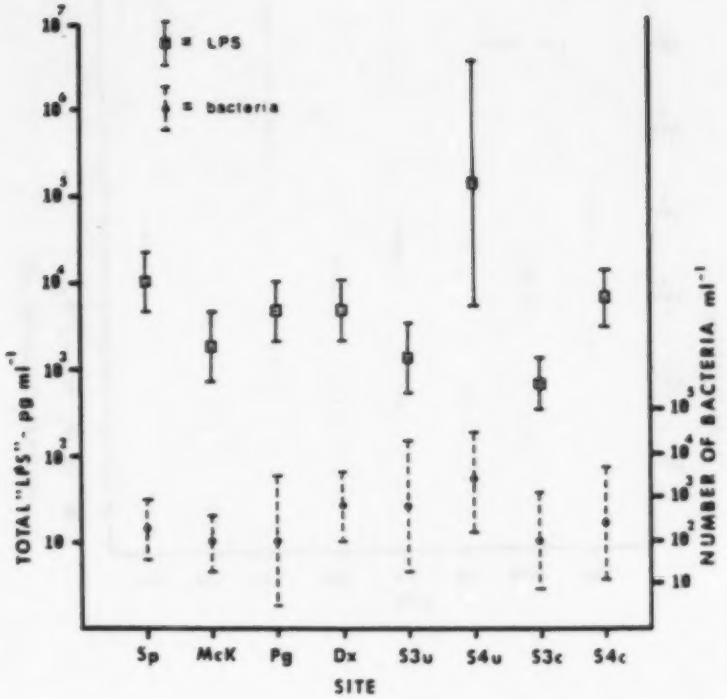

Figure 5. LPS (in picograms-pg) and SPC Bacteria (Incubated for 48 hrs) in Open, Finished Water Reservoirs (Legend as in Fig. 4)

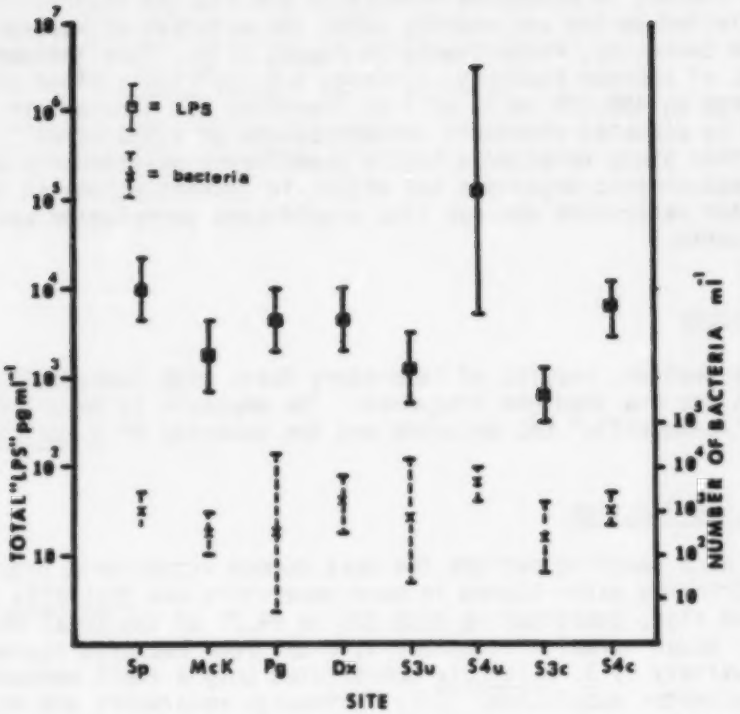

Figure 6. LPS (in picograms-pg) and SPC Bacteria (Incubated for 8 days) in Open, Finished Water Reservoirs (Legend as in Fig. 4)

average algal counts in individual reservoirs are almost exactly paralleled by the average endotoxin concentrations (Fig. 4). On the other hand, the relationship between the mean standard plate counts (2 days and 8 days incubation) is not always evident. At one locality (Pittsburgh reservoir) an increase average endotoxin concentration was observed concurrently with a decrease in SPC bacteria incubated at both temperatures (Fig. 5 and 6).

The blue-green bacteria found in open finished water reservoirs in measurable quantity were represented by S. calcicola. The only time this species was present in excessive numbers in the studied reservoirs was in samples collected during and shortly after the outbreak of waterborne gastroenteritis in Sewickley, Pennsylvania in August 1975. This incident was characterized as of unknown etiology, although a S. calcicola bloom occurred at levels as high as 400,000 cells ml^{-1} in Sewickley No. 4 reservoir. This was accompanied by elevated endotoxin concentrations of 2,500 ng ml^{-1}. The results of this study revealed a highly significant relationship between the density of autotrophic organisms and endotoxin concentrations in open, finished water reservoirs whereas less significant correlation was observed for SPC bacteria.

LABORATORY STUDY

In this section, results of laboratory tests with "unialgal" phytoplankton cultures are analyzed and discussed. The emphasis is on detection of the sources of "nonspecific" LAL gelation and the toxicity of S. calcicola to mice.

Nonspecific LAL Reaction

During both sampling periods the most common autotrophic organism found in treated drinking water stored in open reservoirs was Chlorella vulgaris, a small green alga, constituting from 23% to 99.7% of the total phytoplankton population. Under normal circumstances, blue-green bacteria represented almost exclusively by S. calcicola constituted only a small percentage of the total phytoplankton population. Only Pittsburgh reservoirs and McKeesport reservoirs contained substantial number of S. calcicola (Tables 11 and 13). Therefore, it has been theorized that a nonspecific reaction caused by C. vulgaris and/or other eukaryotic algae may have been responsible for LAL gelation.

Nonspecific reactions of Limulus lysate were described for a number of organic compounds by several authors. It has been shown by Wildfeuer (1974) that gelation of LAL is induced not only by endotoxin of gram-negative bacteria but also by peptidoglycan of various gram-positive organisms. Rotta (1975) has described the endotoxin-like properties of gram-positive bacterial peptidoglycan. Serious implications of bacterial infection such as shock, disseminated intravascular coagulation and Waterhouse-Friederichsen syndrome, generally ascribed to the action of gram-negative bacterial endotoxin, have also been observed in patients with gram-positive infections (Wildfeuer, et al., 1975). Suzuki (1977) indicated that a variety of polysaccharide derivations such as palmitoyldextran phosphate, palmitoyldextran and dextran

phosphate give a positive Limulus lysate gelation, whereas dextran itself was negative (Table 20).

TABLE 20. SUBSTANCES SUSPECTED OF CAUSING LIMULUS LYSATE GELATION

Positive Reaction	Reaction Eliminated By	Negative Reaction
Lipopolysaccharide		Dextran
Glycolipid	mild alkaline hydrolysis	Chitosan
Lipid A (solubilized by BSA and triethyl-amine)		Lentinan
		Starch
		Sucrose fatty acids esters
		Whole blood
Thrombin		Plasma
Thromboplastin		Calcium
		Streptolysin
Poly (A):Poly (U)	ribonuclease	Streptokinase
Poly (I):Poly (C)	treatment	Streptodornase
		Cl. tetani toxin
Peptidoglycan	lysozyme	Hemoglobin
	treatment	Serotonin
		Histamine
		Epinephrine
		Norepinephrine
		Bradykinin
Dextran phosphate		Str. pyogenes
Palmitoyldextranphosphate		Str. pneumoniae
Palmitoyldextran		Staph. aureus

-Compiled from Suzuki et al. (1977), Rotta (1975) and Wildfeuer et al. (1975).

Stanier and Cohen-Bazier (1977) have shown in their thorough review that the cyanobacterial cells contain a peptidoglycan layer which varies in width with different species. This layer may be considerably thicker in many blue-green bacteria than in gram-negative bacteria. The peptidoglycans, LPS and possibly some similar compounds could be effectively removed or destroyed using various chemical treatments (Table 20).

Table 21 shows the gelation of Limulus lysate reacting with C. vulgaris and S. calcicola "in vitro" using intact as well as lysozyme and alkali treated samples. Both species of untreated algae caused LAL gelation at a concentration of 10^5 cells ml^{-1}. The treatment with 0.1 N NaOH completely destroyed the gelling potency of S. calcicola while the same alkali application had no effect on C. vulgaris. On the other hand, the lysozyme treated S. calcicola retained gelation capacity, while lysozyme treated C. vulgaris did not induce LAL gelation. Thus, the lysozyme treatment destroyed the gelation activity of Chlorella suggesting nonspecific Limulus lysate

amoebocyte reaction. The negative results obtained from alkali treated S. calcicola indicated the presence of true endotoxin (lipopolysaccharides) in this species of blue-green alga.

The peptidoglycans and possible related compounds could be effectively removed by lysozyme treatment while most of the LPS is destroyed by strong alkali (such as sodium hydroxide) hydrolysis. In spite of the fact that the lysozyme treatment effectively removes the LAL gelation capacity from C. vulgaris, it has not yet been established what type of compound is responsible for the nonspecific LAL reaction caused by this species. The peptidoglycans were not found in membranes of eukaryotic organisms; however, some other lysozyme-degradable substances in the gelatinous envelopes of green algae may be responsible for the positive LAL reaction.

TABLE 21. LIMULUS LYSATE GELATION OF TWO ALGAL CULTURES

Material (No. of cells ml^{-1})	No pretreatment	Lysozyme treated	NaOH treated
Schizothrix calcicola			
5.5×10^7	++	++	-
5.5×10^6	++	++	-
5.5×10^5	+	+	-
5.5×10^4	-	-	-
5.5×10^3	-	-	-
Chlorella vulgaris			
3.4×10^7	++	+	++
3.4×10^6	++	-	++
3.4×10^5	+	-	+
3.4×10^4	-	-	-
3.4×10^3	-	-	-

++ = firm clot
+ = clot breaks
- = no reaction

The laboratory results also indicate that the sensitivity of LAL for detection of algae is marginal when the counts decrease below 10^5 organisms ml^{-1}. However, the analysis of field samples suggest that the algae may influence LAL gelation even at levels close to 10^4 cells ml^{-1} (Tables 12 and 15). In order to determine the exact predictive value of the assay bound and free endotoxins (or other compounds causing gelation), and the spectrophotometric technique should be used to provide a more accurate representation of gelation that is contributed directly by the living organisms present in the

water sample.

This study strongly suggests that C. vulgaris is the causative factor for LAL gelation in samples from uncovered finished water reservoirs. Therefore, the previous studies (DiLuzio and Friedman, 1973; Jorgensen et al., 1976) on the occurrence of endotoxin in drinking water may have been affected by nonspecific reactions caused by various organic compounds other than "true" endotoxin. In spite of its deficiencies, LAL gelation is a good indicator of "general contamination" (including algae, bacteria and their by-products) of drinking water supplies. Moreover, it can provide reliable results in a relatively short period of time (approx. one hour). Thus, LAL gelation can be a useful tool for tracing sources of contamination in waterborne disease investigations. In order to increase the accuracy and predictive value of LAL test, free and bound endotoxins should be measured (Jorgensen 1976, 1979). Furthermore, the use of alkali and lysozyme treatment is recommended to distinguish between nonspecific LAL reaction produced by eukaryotic algae and/or gram positive bacteria, and "true" endotoxins.

Toxicity of S. calcicola

Intraperitoneal injection

The results of intraperitoneal injections into mice are summarized in Table 22. Schizothrix calcicola was toxic to mice with LD_{50} changing with the age of the culture and ranging from 90-385 mg kg^{-1}. High toxicities were usually caused by young Cyanobacterial cultures (4 days old) while lower toxicity was obtained from older organisms. The toxin could be characterized as typical slow-death-factor (SDF) which caused death in mice within 36 hours, preceded by piloerection, dyspnea, and lethargy. Mortality was completely absent in all control groups. The survival times and physiological effects of SDF were indistinguishable from those observed for Microcystis aeruginosa (Gorham, 1962).

TABLE 22. SUMMARY OF LD_{50} EXPERIMENTS WITH SCHIZOTHRIX CALCICOLA (S.C.) AND ANABAENA FLOS-AQUAE (A.B.) TESTED BY IP INJECTIONS IN FEMALE MICE

Species	Age of culture days	LD50 dry weight-mg/kg	N /number of tests/	Special conditions
S.C.	4	90	1	--
S.C.	8	160	2	--
S.C.	14	132-385	4	--
S.C.	30	230	1	--
A.B.	8	150	1	--
S.C.	8	87	1	Pretreated with phenol and antibiotics
S.C.	8	200	1	Pretreated twice with antibiotics

In order to identify the source of SDF the concentrated S. calcicola cells were treated with a mixture of antibiotics composed of Penicillin G, Chloramphenicol, and Streptomycin for 45 minutes to remove the heterotrophic bacteria. The harvested cultures were also treated with phenol as described by Charmichael and Gorham (1974). The cell concentrate was washed four times using a centrifuge. However, even thorough treatment did not remove all the bacteria and the SPC plates used to estimate the number of bacteria present in S. calcicola cultures indicated contamination even after vigorous treatment by antibiotics and phenol.

Storage of S. calcicola in the freezer reduced the toxicity in several instances. According to our experience this species has to be stored at temperatures below freezing for at least 14 days to remove the toxic effect. Antoclaving and lyophilization destroys the toxin completely.

The results suggest that the toxin produced by S. calcicola is either heat labile or is produced by bacteria closely associated with this species. Similar phenomenon has been described before by Gorham (1962) who observed a toxin associated with accompanying bacteria in Microcystis aeruginosa.

Intubation Experiments

Fourteen tests using four mice per concentration and five tests utilizing eight mice per dose were performed with concentrations ranging from 55 to 180 mg of dry weight per mouse. The highest dose corresponded to 9 g kg^{-1} and was so thick that it was almost impossible to eject it from the syringe. Therefore, it was not technically feasible to test concentrations higher than 200 mg of dry weight per mouse. Out of the 56 mice intubated with S. calcicola cultures of different age, a total of eleven animals died, whereas three mice of 48 died in the controls. The 16-day old culture did not produce any mortality and the tests performed with 4-day old S. calcicola cultures produced negative results as well. The 8-day and 12-day old cultures of S. calcicola caused mortality of 50% and 12% of all animals tested in these groups, respectively. The death distribution was inconsistent, suggesting very low toxicity of S. calcicola to mice when intubated into the stomach. Most of the animals died within 24 hours and the highest mortality was recorded at 6 g kg^{-1}. This elevated mortality in one of the concentrations, however, suggests possible effect on the organism which may cause illness in humans. Although mortality of experimental test animals was recorded, the data was insufficiently distributed for LD_{50} calculation.

Another indicator of toxicity investigated by the laboratory experiments was change in body weight measured in daily intervals for seven days following intubation with fresh, whole cell culture. The hierarchy analysis of variance of the weight change did not reveal any significant difference between the animals within each control, between all three controls and between control mice and animals intubated with S. calcicola cultures. No significant difference in weight change was found when the effect of cultures of different ages was compared and evaluated.

In order to compare the effect of IP injection and intubation on mice, two additional experiments were performed. The focus was on the comparison

of autopsy results using 4 and 8 day old cultures of S. calcicola.

Only one dose (195 mg/mouse = 9.75 g kg^{-1} dry weight) of 4-day old culture was intubated into 8 mice. Subsequent serial dilutions performed with identical cell material were used for IP injections resulting in the following dry weights: 65 mg/mouse (3.3 g kg^{-1}), 32.5 mg/mouse (1.6 g kg^{-1}) and 16.5 mg/mouse (0.8 g kg^{-1}). The 8-day old cultures were also intubated into eight mice (200 mg/per mouse) with appropriate fractions prepared for IP injections (66 mg, 33 mg and 16.5 mg per mouse). Four mice were used for each IP concentration. High mortality (75-100%) was recorded on all injected mice while intubation did not result in any mortality.

The autopsy revealed extensive staining of mesentery by the red pigment derived from S. calcicola. The inflamation and ruptures of blood vessels in mesentery increased with dose. Pericardial hemorrhage and thoracic exudate were also observed in all dead animals. However, this pericardial bleeding seemingly did not increase with dose in mice which were injected with 4-day old culture. On the other hand, the mice injected with 8-day old culture showed the same symptom increasing with the increasing concentration. The liver was always the most affected organ with hemorrhaging nodules and lines on the surface and the damage always increased with the dose. The spleen exhibited hemorrhages similar to those in the liver. The lungs on the intubated mice also exhibited hemorrhagic symptoms. In the lungs of mice injected with S. calcicola the bleeding decreased with increased concentration indicating that the animals died before the lungs were affected. The kidneys were the last organ affected. The autopsy results of the IP group were similar to the intubation group except that the mice injected with S. calcicola have shown heavier staining of internal organs.

Positive Control - Anabaena flos-aquae

Toxic strain NRC-44 of Anabaena flos-aquae was used as a positive control for intubation and IP injection. The toxicity of this species when injected intraperitoneally and evaluated on the basis of LD_{50} (150 mg kg^{-1}) is almost identical to that of S. calcicola. However, the injection of fresh or lyophilized culture of A. flos-aquae into mice caused death in several minutes (usually two-to-ten) preceded by paralysis, tremors, and convulsions which is typical for Anatoxin-A poisoning.

Similar symptoms were observed when concentrated cells were intubated. There was no basic difference in toxicity between fresh and lyophilized cells intubated into mice. The toxicity of ingested A. flos-aquae is approximately ten times lower than that of injected cells (Table 22, Fig. 7).

Cell counts of Anabaena cultures revealed that 1 mg of dry weight contains approximately 6×10^7 cells. This suggests that the LD_{50} value (1.7 - 1.8 g kg^{-1}) for ingested A. flos-aquae is equal to 10^{10} cells. Therefore, in order to achieve mortality relatively high concentration of cells is required to produce the effect. However, diarrhea or gastroenteritis could be certainly caused by much lower dose. Further, the effect of the supernatant and the accumulation of algal or Cyanobacterial products in the water exists and has been demonstrated on several occasions. For instance Gorham

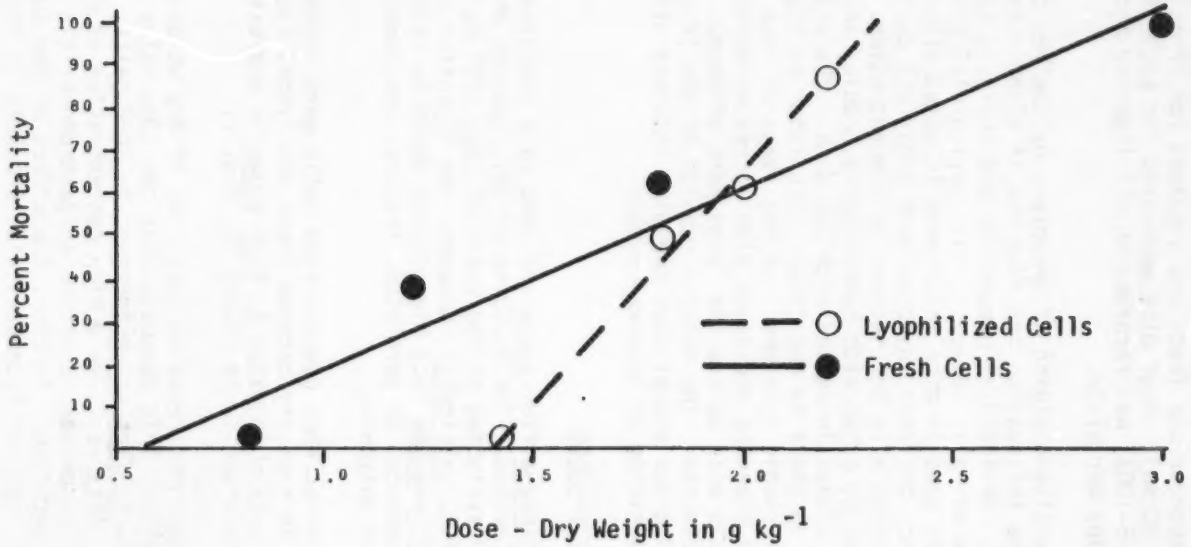

Figure 7. Toxicity of Fresh and Lyophilized Cells of Anabaena flos-aquae Intubated into Mice

(1964) has shown that intraperitoneal injection of filtrates from A. flos-aquae into mice elicit the same effect as the organisms itself.

Endotoxin Isolation

All the laboratory tests including toxicity to mice were performed using the purified, brown ("Sewickley") strain of S. calcicola. However, the extraction and isolation of endotoxin were completed before this brown strain was available. Therefore, the green strain No. 1937 was used for the endotoxin extraction and isolation.

The hot phenol extract technique was used for isolation of crude LPS from the green strain. Beginning with 20g wet-weight of whole cells and following the procedure outlined in the Materials and Methods, 105 mg of crude "suspected" endotoxin were obtained. Initial gas chromatography of this lyophilized sample revealed that lipopolysaccharides were, indeed, present in this substance. Final chemical analyses of the purified lipopolysaccharide revealed the presence of several sugars in the sample together with low quantity of fatty acids. This preliminary analysis suggested that the endotoxin or similar compound is present not only in the brown strain (Keleti et al., 1979) but also in green strains of S. calcicola. To verify this, additional experimentation is necessary with larger quantities of cellular material obtained from S. calcicola.

BIBLIOGRAPHY

Aziz, K.M.S. 1974. Diarrhea Toxin Obtained from a Waterbloom - Producing Species, Microcystis aeruginosa Kuetzing. Science 163:1206-7.

Bailey, S.W. and E.C. Lippy. 1978. Should All Finished Water Reservoirs Be Covered? Public Works Magazine, April issue, 1978, 4 pp.

Banner, A.H. 1966. Marine Toxins from the Pacific, I. Advances in the investigation of Fish Toxins. In F.E. Russel and P.R. Saunders (eds.), Animal toxins. Pergamon Press, New York.

Buttke, T.M. and L.O. Ingram. 1975. Comparison of Lipopolysaccharides from Agmenellum quadruplicatum to Escherichia coli and Salmonella typhimurium by Using Thin Layer Chromatography. J. of Bact. 124:1566-1573.

Carmichael, W.W. and P.R. Gorham. 1974. An Improved Method for Obtaining Axenic Clones of Planktonic Blue-green Algae. J. of Phycol. 10:238-240.

Dean, A.G. and T.C. Jones. 1972. Seasonal Gastroenteritis and Malabsorption at an American Military Base in the Philippines. Amer Jour. of Epidemiology 95:111-127.

DiLuzio, N.R. and T.J. Friedman. 1973. Bacterial Endotoxins in the Environment. Nature (London) 244:49-51.

Drouet, F. 1963. Ecophenes of Schizothrix calcicola (Oscillatoriaceae). Proc. Acad. Natural Sciences of Philadelphia, 115:261-281.

Environmental Protection Agency. 1975. Interim Primary Drinking Water Standards. Federal Register Vol. 40, No. 51.

Evans, T.M., J.E. Schillinger, and D.G. Stuart. 1978. Rapid Determination of Bacteriological Water Quality by Using Limulus Lysate. Appl. Envir. Microbiol. 35:376-382.

Finegold, S.M., W.J. Martin and E.G. Scott. 1978. Bailey and Scott's Diagnostic Microbiology. The C.V. Mosby Company, St. Louis, 514 pp.

Gorham, P.R. 1962. Laboratory Studies on the Toxins Produced by Waterblooms of Blue-green Algae. Am. J. Public Health 52:2100-2105.

Gorham, P.R. 1964. Toxic Algae as a Public Health Hazard. Jour. Amer. Water Works Assoc. 56 (11): 1481-88.

Gupta, R.S. and M.S. Dashora. 1977. Algal Pollutants and Potable Water. In R.B. Pajasek (ed.), Drinking Water Quality Enhancement through Source Protection, Ann Arbor, 431-459.

Herman, L.G. 1976. Sources of the slow growing pigmented water bacteria. Health Lab. Science 13:5-10.

Hindman, S.H., M.S. Favero and A. Peterson. 1975. Pyrogenic Reactions during Haemodialysis Caused by Extramural Endotoxins. The Lancet 18:732-737.

Hoadley, A.W. 1977. Potential Health Hazards Associated with Pseudomonas aeruginosa in Water. In A.W. Hoadley and B.J. Dutka (eds.), Bacterial Indicators/Health Hazards Associated with Water. ASTM Special Technical Publication in 635, ASTM, Philadelphia, 80-114.

James, D.E. 1974. Culturing algae. Carolina Biological Supply Co. 23 pp.

Jorgensen, J.H. 1979. Limulus Amoebocyte Lysate Test to Assess Water Quality. Presented at 79th Annual Meeting of the American Society for Microbiology, May 4-8, 1979, Los Angeles.

Jorgensen, J.H., L.C. Lee and H.R. Pahren. 1976. Rapid Detection of Bacterial Endotoxins in Drinking Water and Renovated Wastewater. Appl. Envir. Microbiol. 32:347-51.

Jorgensen, J.H. and R.F. Smith. 1973. Preparation, Sensitivity and Specificity of Limulus Lysate for Endotoxin Assay. Appl. Microbiol. 26:43-48.

Jorgensen, J.H. and R. F. Smith. 1974. Measurement of Bound and Free Endotoxin by the Limulus Assay. Proc. Soc. Exp. Biol. Med. 146-1042-31.

Keleti, G. and W.H. Lederer. 1974. Handbook of Micromethods for the Biological Sciences. Van Nostrand Reinhold Co., New York.

Keleti, G., J.L. Sykora, E.C. Lippy and M.A. Shapiro. 1979. Composition and Biological Properties of Lipopolysaccharides Isolated from Schizothrix calcicola (Ag.) Gomont (Cyanobacteria). Appl. Envir. Microb. 38:471-477.

Kriess, F.L., Jr. 1977. An Analysis of Endotoxin Levels in Selected Allegheny County Municipal Water Supply Systems. M.Sc. Thesis, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa. 62 pp.

Lippy E.C. and J. Erb. 1976. Gastrointestinal Illness at Sewickley, Pa. J. Am. Water Works Assoc. 68:606-610.

Mikheyskaya, L.V., R.G. Ovodova, and S.U. Ovodov. 1977. Isolation and Characterization of Lipopolysaccharides from Cell Walls of Blue-green Algae of the Genus Phormidium. J. of Bact. 130:1-3.

MMWR-Morbidity and Mortality Weekly Reports. 1979. Endotoxic Reactions Associated with the Reuse of Cardiac Catheters - Massachusetts. 28:25-27.

Moore, R.E. 1977. Toxins from Blue-green Algae. Bioscience 27:797-802.

Palmer, C.M. 1959. Algae in Water Supplies. USDHEW Public Health Service. Cincinnati, Ohio.

Reasoner, D.J. and E.E. Geldreich. 1979. New Medium for Enumeration and Subculture of Bacteria from Potable Water. 79th Annual ASM Meeting, May 4-8, 1979, Los Angeles.

Rotta, J. 1975. Endotoxin-like Properties of the Peptidoglycan. Z. Immun. Forsch. 149:230-244.

Schwimmer, M. and D. Schwimmer. 1964. Algae and Medicine. In Algae and Man. D.F. Jackson (ed.) Plenum Press, New York. pp. 368-412.

Schwimmer, M. and D. Schwimmer. 1968. Medical Aspects of Phycology. In D.F. Jackson (ed.), Algae and Man, and the Environment. Syracuse University Press, Syracuse, N.Y., 279-358.

Standard Methods for the Examination of Water and Wastewater. 1975. 14th Edition. American Public Health Association, American Water Works Association, and Water Pollution Control Federation.

Stanier, R.Y. and G. Cohen-Bazire. 1977. Phototrophic Prokaryotes: The Cyanobacteria. Ann. Rev. Microbiol. 31:225-274.

Suzuki, M., T. Mikanu, I. Matsumoto, and S. Suzuki. 1977. Gelation of Limulus Lysate by Synthetic Dextran Derivatives. Microbiol. Immunol. 21:419-425.

Sykora, J.L., R. Deithorn, R. Reisdorf and G. Kay. 1977. A Follow-up Study of the Sewickley Epidemic. Final Report to the Environmental Protection Agency, 150 pp.

Tisdale, E.S. 1931. Epidemic of Intestinal Disorders in Charleston, W.VA., Occurring Simultaneously with Unprecedented Water Supply Conditions. Amer. J. Public Health 23:198.

Veldee, M.V. 1931. Epidemiological Study of Suspected Waterborne Gastro-enteritis. Amer. J. of Public Health 21:1227-1235.

Watson, S.W., T.L. Novitsky, H.L. Quinby and F.W. Valois. 1977. Determination of Bacterial Number and Biomass in the Marine Environment. Appl. Envir. Microbiol. 33:940-946.

Weckesser, J., A. Katz, G. Drews, H. Mayer and I. Fromme. 1974. Lipopolysaccharide Containing L-acofriose in the Filamentous Blue-green Alga Anabaena variabilis. J. Bact. 120:672-678.

Weise, G., G. Drews, B. Jann and K. Jann. 1970. Identification and Analysis of a Lipopolysaccharide in Cell Walls of the Blue-green Alga Anacystis nidulans. Arch. Mikrobiol. 71:89-98.

Westphal, O. and K. Jann. 1965. Bacterial Lipopolysaccharides, Extraction with Phenol Water and further Applications of the Procedure. In R.L. Whistler and M.L. Wolfrom (eds.). Methods in Carbohydrate Chemistry 5:83-91.

Wildfeuer, A., B. Heymer, K.H. Schliefer, P.H. Seidel and O. Haferkamp. 1974. Zur Schockdiagnostik: Der Nachweis von Endotoxin und Mucopeptid mit dem Limulus polyphemus-Lysat-Test. Klin. Wschr 52:175-178.

Wildfeuer, A., B. Heymer, D. Spilker, K.H. Schliefer, E. Vanek and O. Haferkamp. 1975. Use of Limulus Assay to Compare the Biological Activity of Peptidoglycan and Endotoxin. Z. Immun. Forsch. 149:258-264.